Chronic nitrogen and nutrient deposition impacts on community structure and abundance of bacteria, fungi, and CH_4 cycling prokaryotes in a northern peat bog

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ACCESSIBILITY CLAUSE AND PERMISSION TO USE

I. Abstract

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Anthropogenic nitrogen (N) deposition may threaten peatland carbon storage capabilities. Increased deposition has been linked to subsequent plant community shifts and increased decomposition rates, potentially via the disruption of natural microbial communities. By examining peat soils from randomized, replicated treatment plots in a long-term simulated chronic N deposition experiment at the Mer Bleue Bog in Eastern Ontario, Canada, my objective was to characterize how increased deposition impacts community structure and abundance of broad groups of microbial communities, and specific CH_4 cycling prokaryotes. Using fingerprinting approaches and qPCR of SSU rRNA and other functional genes, my data show that with increasing nutrient loading the bacterial and fungal community structure changed. Along the same gradient methanogen abundance decreased, however there were no corresponding changes in methanotroph community structure or abundance. My results provide new insights on the possible causes of higher CO_2 and CH_4 effluxes seen *in situ* following chronic nutrient loading.

Keywords

Peatland, Mer Bleue, nitrogen deposition, microbial community, bacteria, fungi, T-RFLP, qPCR, pyrosequencing, *pmoA*, *mmoX*, *mcrA*, methanotroph, methanogen, methane monooxygenase

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1. Introduction

Northern peatlands are important long term carbon (C) sinks as a result of a net primary production exceeding organic matter decomposition. In fact, despite that peatlands cover only 3% of Earth surface they have stored approximately one third of the amount of C in the atmosphere (approx. 450 Gt) in Earth's soils since the beginning of the Holocene; with long-term rate of C accumulation of up to 30 g C m^{-2} yr^{-1} (Gorham, 1991; Turunen et al., 2002). Climate and hydrological factors contribute to this net imbalance between primary production and decomposition. Soils are typically cool at depth even in the growing season, which keeps metabolic rates low. Peatlands are also generally wet, creating an anoxic layer in the soil profile that leads to reduced conditions that and promote fermentation reactions and methanogenesis as the slow drivers of decomposition. Thus, peatlands play important roles in the global climate system through the exchange of both CO_2 and CH_4. The past and current function as C sinks is uncertain with current direct anthropogenic effects via drainage or mining, or indirect effects through broader climate (drought, warming, higher CO_2 concentrations) and other environmental changes including atmospheric pollution deposition (Limpens et al., 2011). Emissions of reactive nitrogen (N) (i.e. NO_x and resulting HNO_3, and NH_3^+) due to fossil fuel combustion and agricultural intensification have disrupted the input of available N to ecosystems in general; anthropogenic production has increased 9 fold in the last century (Galloway and Cowling, 2009). N and nutrient availability in peatlands may increase in other ways as well; while greenhouse gases rise, increases in temperature and altered precipitation can speed decomposition of stored organic matter and release N and other nutrients in available forms in the soil profile. This temperature effect has been shown to be comparable to an experimental application of almost 4 g N m^{-2} yr^{-1} for each 1°C increases (Limpens et al., 2011).

Sphagnum mosses tolerate low nutrient conditions in bogs and poor fens and obtain their N and other nutrients from their own older tissues (Rydin and Clymo, 1989); this largely circumvents the need for N from decomposition of organic matter. *Sphagnum* mosses generally render soil inhospitable to microbes via recalcitrant litter and acidity generation and tight conservation of N. These factors also allow the *Sphagnum* mosses to outcompete many vascular plants, which rely more heavily on decomposition of soil organic material for nutrient supply (Malmer et al., 2003). However, through anthropogenic activities such as fossil fuel combustion and modern, high-intensity agriculture, reactive atmospheric N input has increased in some peatland region (Galloway et al., 2008). Generally, N addition increases biomass production in terrestrial ecosystems (LeBauer and Treseder, 2008). However, in nutrient poor ecosystems, such as peatlands, increased N input can negatively impact species diversity and ecosystem function. As more N is deposited onto peatlands, *Sphagnum* mosses become N saturated and N is able to reach higher vascular plants roots (Lamers et al., 2000). Eventually vascular plants are able to become dominant. With longer stems and broader foliage, they can outcompete the shorter *Sphagnum* mosses for light (Berendse et al., 2001; Bubier et al., 2007). Increasing N deposition rates have been shown to lead to a progressive shift in vegetation where *Sphagnum* mosses were outcompeted by vascular plants, while gross photosynthesis and net primary production remain unchanged (Larmola et al., 2013). The new plant community assimilates more N and in turn releases N faster after tissue senescence, providing more nutrients to microbial communities and the ecosystem at large (Bragazza et al., 2012; Larmola et al., 2013).

In peatlands under a natural N deposition gradient, an increase in microbial CO_2 production and dissolved organic matter was observed (Bragazza et al., 2006). Additionally, in simulated N loading experiments, peat decomposition has been shown to increase (Basiliko et

al., 2006) and total net ecosystem exchange/C sequestration supported by the dominance of *Sphagnum* mosses, decrease despite that there was an overall increase, or little change in net primary productivity by vascular plants (Bubier et al., 2007; Gunnarsson et al., 2008; Larmola et al., 2013). With the absence of the inhibitory capabilities of *Sphagnum* mosses and increases in nutrient supply from detritus and soil organic matter, the soil biodiversity and functioning of microflora will likely change. Observed increases in decomposition in the few short-to-medium term peatland nutrient deposition loading experiments points to a more diverse microbial community that might help the vascular plants thrive even further; however, these relationships have not yet been examined in detail. Root exudates and better litter quality are factors that can enhance soil fertility, providing more labile C and nutrients to promote microbial growth. Of course in peatlands, where the natural state is often one of nutrient limitation that promotes C sequestration, improved soil fertility will disrupts peatland C storage capabilities.

Globally, soil respiration by roots and soil microbes are the largest source of the flux of C from terrestrial ecosystems to the atmosphere. The Intergovernmental Panel on Climate Change estimates soil respiration releases 107.7 Pg C yr^{-1} relative to photosynthetic inputs of 108.9 Pg C yr^{-1} (IPCC, 2013). This is indicative of some net sequestration in the terrestrial biosphere (especially in systems such as peatlands). Any small increase in global soil respiration could increase microbial output of CO_2 to levels that could equal and even surpass to C inputs (Cox et al., 2000). In the longest-running simulated N loading experiment at the Mer Bleue Bog, Ottawa, Canada, a net increase of 24–32% in ecosystem respiration and a loss of *Sphagnum* moss was observed, which was likely driven by faster decomposition (Juutinen et al., 2010). This is somewhat paradoxical however, as it has also commonly been shown that with increased N loading to forest soils with recalcitrant organic matter, microbial biomass decreases as a results

of N toxicity (Treseder, 2008) or due to formation of stable amino-quinone compounds (Ågren et al., 2001). Specifically in peatlands in the short-term however, increases in nutrients can promote increased microbial biomass (as observed in Mer Bleue bog in after 2 growing seasons; Basiliko et al., 2006). In the same field experiment in subsequent years, microbial activity remained elevated (Larmola et al., 2013). Increasing ecosystem respiration and decomposition rates could possibly mean more diverse and specialized microbes appearing that consume higher quality plant litters.

Beyond cycling of CO_2, peatlands also typically emit methane (CH_4), which is produced in the anoxic soil layers by strictly anaerobic Euryarchaeota. However some to all CH_4 can be oxidized by aerobic CH_4 oxidation bacteria if there is an oxic zone in the peat profile. Per molecule, CH_4 is a more potent greenhouse gas comparative to CO_2, up to 21 times over a 100 year period. The Intergovernmental Panel on Climate Change reports that with a very high level of confidence, that atmospheric CH_4 has increased during the Industrial Era and is caused by anthropogenic activities, and it is accepted to be the 2nd most important greenhouse gas contributing to global warming (IPCC, 2013). Despite anthropogenic increases, natural wetlands are still the largest sources of CH_4 and drivers of interannual variability in atmospheric concentrations (177 to 284 Tg of CH_4 yr^{-1}). Attributes of peatlands including specialized vegetation, water table depth, soil water content, and temperature affect CH_4 production and oxidation. Plant-mediated transport of CH_4 is responsible for 50-95% of CH_4 fluxes in wetlands (Hanson and Hanson, 1996). CH_4 diffuses into the rhizomes and is transported through the plants and to the atmosphere, bypassing the soil surface oxidation of CH_4 by aerobic methanotrophic bacteria. Water table position affects decomposition processes and CH_4 fluxes by largely delineating the anoxic and oxic layers in peat. Moore and Knowles (1989) have observed

experimentally that CH_4 emission rates decreased exponentially as the water table was lowered. Soil water content is also a major control on gas diffusion. Dryer soils have greater oxygen penetration and more rapid transport of CH_4 to soil methanotrophs, consequently, increasing CH_4 oxidation (Hanson and Hanson, 1996). Lastly, temperature has been shown to link to higher CH_4 emissions; with rates higher as soil temperature rises (van Winden et al., 2012). However because there are a range of biotic (production, oxidation and plant transport) and abiotic factors that influence CH_4 emissions from peatlands, we do not have a complete understanding of CH_4 emission feedbacks in response to environmental changes.

Aerobic methanotroph communities typically reside at the oxic-anoxic boundary layer with supplies of atmospheric O_2 from above and with methanogen-generated CH_4 from below. There, methanotrophs consume CH_4 chemoautotrophically with CH_4 (or occasionally other C1 compounds) as the sole source of C and electrons. This group of bacteria generally can be divided into two taxonomic groups. Based on their cell morphology, ultrastructure, phylogeny, and C-assimilation pathways these are defined as type I and type II (Hanson and Hanson, 1996). Type I methanotrophs belong to the gamma subdivision of the *Proteobacteria* and employ the ribulose monophosphate pathway for formaldehyde assimilation. As for Type II methanotroph, they belong to the beta subdivision of the *Proteobacteria* and use the serine pathway for formaldehyde assimilation. Both groups have appear to favor specific conditions respectively. Type I are typically found in conditions where CH_4 is limiting and with high N and copper concentration. Type II are the opposite, growing with low N and copper concentrations but relatively high CH_4 concentration, more typical of nutrient poor peatlands (Hanson and Hanson, 1996). All methanotroph contains key CH_4 monooxygenase (MMO) enzymes that catalyze the

first step of oxidation of CH_4 to methanol as the first step for redox and assimilation of CH_4-C (Hakemian and Rosenzweig, 2007).

CH_4 monooxygenases can occur in two forms: a membrane bound particulate form (pMMO) and cytoplasmic soluble form (sMMO). It is known that copper plays a key role in the physiology and activity of aerobic methanotrophs as well as influencing which form of MMO is produced in some methanotroph species (Semrau et al., 2010; Vorobev et al., 2013). Copper is important in the production of pMMO and is found within its active site and is believe to enhance pMMO activities (Semrau et al., 1995). In environmental conditions where copper is absent, sMMO might be favored. This switch is called the "copper switch" (Semrau et al., 2013). Both MMO forms of enzyme, especially sMMO, have broad substrate specificities and can oxidize alkanes, alkenes, alicyclics, aromatics, ethers, heterocyclics and ammonia (Colby et al., 1977; Dalton, 1977). These processes are forms of co-metabolism, with the methanotroph gaining no energy or C.

The pMMO contains copper and iron and is expressed via a three-gene operon, *pmoCAB*, which code for three integral membrane (Gilbert et al., 2000). *pmoA* is well studied and a large dataset of sequences are available at GenBank from environmental studies and isolates. It provides a useful molecular marker for methanotroph ecology studies. To date, the pMMO enzyme is found in all methanotrophs except the genus *Methylocella*, whereas sMMO is rarer; most methanotrophs rely solely on pMMO (Hanson and Hanson, 1996). The sMMO gene cluster is encoded by a six-gene operon: *mmoXYZ, mmoC, mmoB and mmoD* (gene on another operon) (Cardy et al., 1991). Recently, the sMMO cluster has been sequenced on *Methylococcus capsulatus* (Bath), *Methylosinus trichosporium* OB3b, *Methylosinus sporium, Methylocystis sp.*, *Methylocella silvestris* BL2 *and Methylomonas sp.* which provide a relatively small reference

dataset. Both *pmoA* and *mmoX* have been shown to produce comparable phylogenies with the 16S-based phylogenies of the same organisms (Holmes et al., 1999; Kolb et al., 2003).

In the late 1980s it was observed that the addition of NH_4NO_3 to temperate forest soils inhibits CH_4 uptake (Steudler et al., 1989). Subsequent studies showed this pattern in other soils (Bodelier and Laanbroek, 2004). The reason is likely that ammonia and CH_4 monooxygenases are closely related phylogenetically and MMOs have been shown to bind NH_4^+ over CH_4 preferentially (Murrell et al., 1998). Additionally, the by- products (hydroxylamine and nitrite) of nitrification have shown to be toxic to methanotrophs (Schnell and King, 1996). The understanding of N effects on CH_4 oxidation in wetlands is not entirely clear. Some studies have shown that after one decade following urea addition to rice paddies, CH_4 oxidation conclusively increased due to overcoming simple N limitation for the autotrophic methanotrophs that cannot access organic soil N (Bodelier et al., 2000). In peatlands, few studies have explored how mineral N and other nutrient additions influence methanotrophic bacteria and methanogenic archaea (Bodelier et al., 2000; Bodelier and Laanbroek, 2004).

In summary, with increasing N deposition from anthropogenic activities or indirectly through climate-change enhanced peat mineralization, a surge of available N and other nutrients may be fed into peatland systems. Peatlands have stored an important amount of C, however inadvertent addition of bioavailable nutrients may allow opportunistic plants to change the landscape and in turn drive C fluxes. Either via direct impacts from the N deposition or the indirect effects caused by plant community shifts, soil chemistry will likely change, with improved resources for microbial communities. Microbes may have sufficient energy to breakdown more complex molecules and in turn further mineralize nutrients. As plant communities continue to shift towards leafy vascular plants, the new foliage will provide more

labile energy and C resources for the microbes as a result of litter fall and/or root exudates. This cycle will result in C losses from the ecosystems. . The poorly understood mechanisms of N inhibition of CH_4 oxidation may also contribute to anticipated changes in greenhouse gas cycling. A key concern is that under higher nutrient availability, peatlands will both no longer act as a C sink and might produce and emit more CH_4.

2. MSc thesis objectives

My objective is to characterize microbial communities following simulated chronic N loading (with and without other nutrients) in the Mer Bleue Bog in two steps. (1) I will investigate broad changes in microbial community structure associated with the previously reported shifts in vegetation and litter chemistry over the past 15 years, and characterize abundances of bacteria and fungi and linkages to peat mineralization. (2) I will target specific CH_4 oxidizing bacterial and methanogen communities and activities in the same nutrient loading experiment.

I predict that mineral N added at the Mer Bleue bog will be assimilated quickly by the vegetation. As a result, nutrients provided from plant litters will be more labile, enhancing Chronic N and nutrient deposition impacts on community structure and abundance of bacteria, fungi, and CH4 cycling prokaryotes in a northern peat bog capabilities are weakening (Larmola et al., 2013). Also, I predict that CH_4 monooxygenases will selectively oxidize the ammonia added (and/or naturally mineralized); leading to reduced biomass, diversity and activity of methanotrophs. Both predictions hypothetically would support scenarios of increased atmospheric CO_2 and CH_4 arising from microbial feedbacks in peatlands and further driving climate change. However, ecosystems have many complex feedback mechanisms that we still

do not understand. Looking at the microbial response to N and other nutrient additions to a bog is a first step in understanding the complex feedbacks between increased N pollution and these important systems within the terrestrial biosphere.

2.1 Thesis structure

This thesis consists of two subsequent journal style chapters. Chapter one describes my work on Mer Bleue Bog's bacterial and fungal communities subjected to chronic N and combined nutrient loading, while chapter 2 evaluates the methanotroph and methanogen communities and their potential impact on overall CH_4 dynamics.

3. Chapter 1

3.1 Introduction

Peatlands accounts for just 3% of the Earth's terrestrial surface but have stored more than one third of the amount of carbon (C) currently in the atmosphere over the Holocene epoch (Gorham, 1991). Plant growth exceeds decomposition and thus they are long-term net sinks of atmospheric CO_2. Soil decomposition is slowed or halted by many environmental factors: low pH (Williams et al., 2000), cold (Rydin and Jeglum, 2013), waterlogged anoxia, potential nutrient limitation (Moore and Basiliko, 2006) and high concentrations of phenolic compounds from *Sphagnum* mosses, which are toxic to microbes (Yavitt, 2000; Jassey et al., 2011). Constrained nutrient availability is an important factor among the list. With increasing human activities, fossil fuel combustion and intensive agricultural practices have dramatically increased reactive atmospheric nitrogen (N) concentrations. Through dry or wet deposition, peatlands are fertilized, thereby reducing nutrient limitations to the vegetation community and ultimately increasing plant tissue decomposition (Turunen et al., 2004; Larmola et al., 2013). In bog and poor fen sites that predominate peatlands across Canada, taller and denser vascular plants become more dominant and shade *Sphagnum* mosses under N and combined nutrient loading. As *Sphagnum* mosses are lost, new plant tissues, including belowground roots that are typically more nutrient rich, become important sources of litters to soils (Preston et al., 2012) possibly changing microbial communities. Plant community shifts under increased N have been relatively well documented in both North American and Northern European contexts; however subsequent effects of the vegetation shift on microbial communities and ultimately C losses in nutrient-poor peatlands are not clear.

Initial reports indicated that inorganic N addition to soil organic matter across a large range of ecosystems slows decomposition, particularly with nutrient-poor plant litters (Fog, 1988). Agren et al. (2001) explains these observations through decreased bacterial and fungal growth rate, altered microbial community with increased microbial efficiency and more rapid formation of recalcitrant compounds. However in more recent studies focussing on peat soils, groups have shown the opposite (Basiliko et al., 2006; Bragazza et al., 2006; Larmola et al., 2013). Bragazza et al. (2006) showed that higher atmospheric N deposition in European peatlands has increased CO_2 emissions and dissolved organic C release. They theorized that increasing N availability favored microbial decomposition by removing N limiting constraints on microbial metabolism and promoting a positive feedback of microbial enzymatic activity through better litter quality. Both Basiliko et al. (2006) and Larmola et al. (2013) showed that in a shorter-term and longer fertilization experiment respectively, the increased N impacts microbial communities in a bog via increased mineralization of C and increased microbial biomass, both supporting overall increased decomposition in a nutrient deprived bog ecosystem where decomposition rates are typically very slow. Additionally, loss of *Sphagnum* mosses causes other environmental changes in nutrient poor peatlands. Bogs with high nutrient loading become wetter as the peat layer thinning though relative depression of the peat. Higher litter quality has appeared as a results of the enhancement of shrub biomass and appearance other vascular plant functional types (Wang, Murphy, et al., 2014). Chloroform fumigation-extraction and phospholipid fatty acid analyses have provided coarse-scale microbial biomass and microbial community structure data to help shed light on N loading impacts on peatland microbes (Basiliko et al., 2006). However, there is little detailed information on how fungal and bacterial communities are affected by nutrient loading in peatlands.

Soil microbial communities are highly diverse and complex and fingerprinting methods provide a good overview to assess the community structure of dominant members. Terminal restriction length fragment polymorphism (T-RFLP) is a fingerprinting method based on restriction fragments (digested with nucleases) of target gene of interest, and 16S and 18S small subunit (SSU) ribosomal RNA (rRNA) genes or group-specific functional genes are commonly assayed. T-RFLP is a highly reproducible technique for monitoring spatial and temporal changes and has proven to be effective in differentiating between different microbial communities in different environments (Tiedje et al., 1999; Lukow et al., 2000). Other techniques to understand complex microbial interactions involve dividing them into ecological meaningful groups. Characterizing bacterial and fungal communities both in terms of fingerprinting, but also quantification of gene copies using quantitative polymerase chain reaction (qPCR) can help simplify a complex microbial community and evaluate its dynamics. qPCR is an efficient and rather inexpensive approach to assess microbial dominance in soils when compared to other molecular techniques (Strickland and Rousk, 2010). It does not, however, indicate abundance of these microbial groups as biomasses, only in terms of gene copy numbers. Bacteria and fungi are known to respond differently to nutrient availability in peatlands (Myers et al., 2012), and thus this differentiation is relevant to the current study.

The Mer Bleue Bog, a large ombrotrophic site located east of Ottawa, ON, has been the focus of more than 100 of studies dealing with plant community, hydrology, C and CH_4 exchange, and decomposition. This includes the longest chronic nutrient amendment experiment to assess N and other nutrient impacts on C exchanges. Here, my objective is to characterize the microbial community and its response to chronic nutrient loading at the Mer Bleue Bog in two sampling years (2013 and 2014). I characterized the microbial community structure using T-

RFLP and the relative proportions of fungi to bacteria ratio using a qPCR. This built on the work of Larmola et al. (2013), who at Mer Bleue demonstrated that higher ecosystem-level respiration was counteracting increased net primary production under enhanced N and combined nutrient loading. My data should reflect changes in vegetation shift shown by Larmola et al. (2013). Moreover, given the length of the nutrient loading and substantial plant-community, hydrological, and soil chemical changes seen, treatment effects on microbial communities should not change much from one sampling year to the next.

3.2 Methods

3.2.1 Experimental site and sampling

The Mer Bleue bog (45°24' N latitude, 75°31' W longitude), located 10 km east of Ottawa, Canada, is a raised ombrotrophic peat bog complex with hummock-hollow patterned topography. It is mainly *Sphagnum*-dominated with a shrub overstory. The water table depth averages 30 cm beneath the surface of the hummocks. Background N deposition is relatively high to other locations in Canada with an approximate total annual deposition of 1.5 N g m^{-2} yr^{-1}. Regional wet atmospheric N deposition was estimated to be 0.6 to 0.8 g N m^2 yr^{-1} (Basiliko et al., 2006).

Field fertilization experiments were established in 2000-2001 (Bubier et al., 2007). Fertilization plots (9 m^2 in area) were set up in a 400 m^2 section of the bog with little variation in micro-topography and vegetation. Solutions of N were added every three weeks as ammonium nitrate (NH_4NO_3) at 1.6**,** 3.2 and 6.4 g N m^{-2} yr^{-1}, approximately 5, 10 and 20 times the ambient growing season atmospheric deposition. Phosphorus and potassium (PK) were added as KH_2PO_4, equivalent to 6.3 g K and 5.0 g P m^{-2} yr^{-1}. Control plots received P and K solution or distilled

H_2O. Each treatment was established in triplicate and from here on, treatments are identified as Control, PK, 5N, 5N+PK, 10N, 10N+PK, 20N and 20N+PK.

Sampling strategies for 2013 and 2014 are described in Table 3-1. In mid-August, 2013, for each plot, three cores were taken using a Russian corer that was washed with distilled water between plots. Soil 5-15 cm below the live vegetation was sampled and stored in a sterile plastic sampling bag. Each bag was homogenized by mixing the sample by hand. Sample bags were placed in a cooler with ice until they were transported to Laurentian University where they were stored at 4°C.

Samples were sent to analytical laboratories (Soil and Plant Analytical Lab at the Great Lakes Forestry Centre in Sault Ste.Marie, Ontario) for analysis of total elemental concentrations. Total C and N were determined using a solid phase combustion analyzer while other elements were measured using the EPA 3051A acid digestion protocol and then determined using a Varian ICP-OES spectrometer.

3.2.2 Terminal restriction fragment length polymorphism (T-RFLP) and quantitative PCR (qPCR)

DNA was extracted from peat samples with the PowerSoil® DNA Isolation kit (MO BIO Laboratories Inc., Carlsbad, CA). All DNA samples used in subsequent experiments were pooled from three extractions. Homogenization was performed using a 5 minute cycle on a 16 tube MiniBeadbeater TM (Biospec Products Inc., Bartlesville, OK). Samples were stored at -20°C for analysis at a later date.

PCR reactions were performed using an MWG AG Biotech Primus 96+ Thermocycler. Previously determined optimized primer combinations and cycles from Preston et al. (2012)

were utilized for the amplification of bacterial 16S and fungal 18S ribosomal RNA genes. Bacterial and fungal T-RFLP was performed as described by Preston et al., (2012). Fragment size analysis was completed at the Agriculture and Food Laboratory at the University of Guelph (Guelph, ON). T-RFLP data were preprocessed in R using a custom function created using the algorithm described by Ishii, Kadota and Senoo (2009) with slight modifications to accommodate the data format at hand. The cut-off distance was set to 2 bp and the final output was expressed as proportion total peak height per T-RF by sample (Kaplan and Kitts, 2003).

The bacterial and fungal SSU rRNA genes were qPCR-amplified using oligonucleotide primers that broadly targets members of the domain Bacteria and the kingdom Fungi following methods described in (Fierer et al., 2005). qPCR assays were conducted in polypropylene 96–well plates on an Agilent Technologies Stratagene MX3005P qPCR system. Each 20 µl reaction contained the following: 10 µl of Thermo Scientific DyNAmo HS SYBR green qPCR 2X Master Mix, 0.5 µl of each primer sets 9 µl of dH_2O. PCR conditions for bacterial SSU rRNA were 15 min at 95°C, followed by 40 cycles of 95°C for 15 sec, 30 sec at 50°C for Bacteria and 53°C for Fungi, and 72°C for 1 min. All reactions including sets of standards were performed in triplicate. After each qPCR run, melting curve analysis was performed to verify the presence of the desired amplicon and not from primer-dimers or other artifacts. Data collected are described as an arbitrary relative copy number over g of wet soil (data are normalised).

3.2.3 Statistical analyses

3.2.3.1 qPCR analysis

Standard curves were produced using triplicate 10-fold dilutions of DNA from pure culture *E.coli* for Bacteria and *S. cerevisiae* for Fungi. At least three nonzero standard

concentrations per assay were used with concentration ranging from 10^{-2} to 10 ng of DNA per reaction. Target copy numbers were calculated from the standard curves using threshold cycle value (CT). For all qPCR assays, there was a linear relationship between the log of the DNA copy number and the CT across the specified concentration range ($R^2 > 0.95$). Amplification efficiencies, calculated using Pfaffl's (2001) formula $E = 10^{[-1/slope]}$, varied from 1.9 to 2.0 across both Bacteria and Fungi qPCR assays. Estimated gene fragment copies were used to calculate the F/B ratio. Linear regression analysis were performed on those ratio compared to N deposition level with and without P and K.

3.2.3.2 T-RFLP-based community analysis

To evaluate microbial richness and evenness, Shannon and Simpson index was performed using vegan package provided by R statistical software. Microbial communities between samples were compared using non-metric multidimensional scaling (NMDS) with the vegan package provided by R statistical software (R Core Team, 2013; Oksanen et al., 2014). Restriction fragment peaks were analyzed in the context of relative abundance as presence or absence. Adonis test using an eigenvalue method was performed to determine whether there were significant differences between treatments (Oksanen et al., 2014). An agglomerative hierarchical cluster analysis based on Ward's method of dissimilarity calculation was used to provide another visual indication on relatedness among the treatments.

3.3 Results

2014 was marked by considerably more precipitation and the water table position in 2014 was significantly higher than in 2013 (Figure 3-1), leading to a higher moisture content in the soil. Chemical analysis of peat samples showed a decrease in C/N ratio as N was added

(Figure 3-2). Total C did not change among treatments however. Total N showed a significant increase between control and N plots with 20 N+PK being significantly different than 20N without the addition of PK ($p = 0.01$; Table 3-2; Figure 3-2).

3.3.1 Quantitative analyses of gene copy numbers and F/B ratios

For all qPCR assays, amplification efficiencies varied from 1.7 to 2.0. A linear relationship between the log of the DNA standards and the calculated threshold cycle value was observed across the diluted concentration ($R^2 > 0.95$). These values were consistent with those reported in the literature (Fierer et al., 2005).

In 2013, fungal 18S rRNA gene copy numbers remained unchanged as N fertilization increased ($p = 0.67$; Figure 3-3). Addition of P and K also had no significant effect ($p = 0.38$). Bacterial 16S rRNA copy number also showed no significant changes as N fertilization increased ($p = 0.54$) and the addition of P and K had no effect ($p = 0.26$). In 2014, fungal 18S rRNA copy numbers remained unchanged with increasing N ($p = 0.17$) and the addition of P and K also had no effect with a $p = 0.14$ (Figure 3-4). Bacterial copy numbers significantly increased with N ($p = 0.007$) there was no effect ($p = 0.57$) with the addition of PK (Figure 3-4).

The Fungi:bacteria ratio in 2013 samples suggest no significant difference ($p = 0.53$, Figure 3-5). The addition of P and K significantly decreased the overall ratio across all different levels of the treatment plots ($p = 0.05$). The following year, the F/B ratio showed a decreasing pattern with increasing N additions, although not statistically significant ($p = 0.21$; Figure 3-6). The addition of P and K decreased the F/B ratios on average but differences between N fertilization alone and with the addition of P and K were not significant ($p = 0.13$).

3.3.2 Microbial community structure

Shannon indices of both fungi and bacteria combined were calculated and showed no significant difference between the different treatments in either year . Similarly for each microbial group alone there were also no significant differences (Table 3-3). No pattern were also observed for Simpson indices

To visualize community structure differences among treatments, NMDS of 2013T-RFLP data revealed groupings of Controls separated from high N fertilization plots (20N + PK) (Figure 3-7). Additionally, hierarchical clustering using Ward's method also presented a similar pattern as the NMDS (Figure 3-7). However, using adonis testing, the differences were not significant ($p < 0.05$; Table 3-4). Similar patterns were observed in 2014 and adonis testing showed significant differences (Figure 3-10, Table 3-4).

When observing the microbial groups individually, bacterial communities in 2013 and 2014 were significantly different between control and low N plots compared to high N plots using both adonis tests and as visualized via multivariate techniques (Table 3-4; Figure 3-8; Figure 3-11. As for fungal communities, in 2013, there were no significant differences between control and low N plots versus high N plots as depicted in the NMDS and cluster analysis ($p = 0.34$; Table 3-4; Figure 3-9. In 2014, fungal communities were significantly different between control and low N treatments relative to high N treatments (Table 3-4; Figure 3-12). Addition of PK did not affect the fungal community composition.

3.4 Discussion

Although my T-RFLP data do not provide specific taxonomical information on microbial groups, they do indicate overall broad changes to microbial community structure. My data suggest that there are significant microbial community changes as N deposition increases, but not

at low N addition rates (Figure 3-7; Figure 3-10). These data build on patterns seen in two previous studies at Mer Bleue (Basiliko et al., 2006; Larmola et al., 2013). Both studies showed higher level of microbial respiration rate in the 20N+PK plots relative to control. In 2013, changes in microbial communities could be attributed to altered community structure (with addition of P and K) as adonis tests indicated significant changes in the bacterial community structure (Table 3-4, Figure 3-8) and also shown in my qPCR data (Figure 3-3). In 2014, changes in microbial communities were attributed to both bacterial and fungal community structure (Table 3-4; Figure 3-4; Figure 3-11; Figure 3-12). Based on qPCR data, the possible increase in decomposition could be the results of an increase in bacterial numbers and bacterial community diversity (Figure 3-8; Figure 3-3). Peat C/N ratios appeared to be decreasing, which fits conceptually with additional N inputs, but the pattern was not statistically significant ($P < 0.11$), primarily due to the variability between replicate plots. As the C/N ratio drops from 52.21 to 33.93 (Average C/N ratio of Control and 20N+PK plots respectively), the soil condition becomes more favorable for microbial decomposition (Manzoni et al., 2008). N deposition increases, the nutrient poor bog is slowly becoming more nutrient rich, supporting bacteria that have higher biomass N requirements and that are superior competitors in terms of C mineralization over fungi in nutrient rich peatlands (Myers et al., 2012).

Atmospheric N deposition onto low nutrient soil such as in peatlands has been shown to decrease mycorrhizal fungal species richness (Lilleskov et al., 2011). My data indicate where high N+PK plots have decreased fungal diversity compared to controls in 2014 (Figure 3-4). Perhaps N added as NH_4 and NO_3^- allowed plants to uptake the nutrient without the help of fungal partner to access organic N sources that predominate in unimpacted bogs and poor fens (Lilleskov et al., 2002; Hasselquist et al., 2012). Mycorrhizal fungi are abundant in soils with

low N availability and are known to form symbiotic relationships with *Ericaceae*, which are abundant in the higher N plots (with and without P and K) (Selosse et al., 2007; Larmola et al., 2013). Additionally, dark septate endophytic fungi might be important in control and low N plots as they contribute to nutrient and C cycling within the plant hosts; they produce the extracellular enzyme machinery required to breakdown complex plant polymers, including phenolic compounds (Caldwell et al., 2000). As vegetation cover changes and nutrients become available, the endophytic fungi might not be needed. The decreasing fungal numbers could be a result in loss of mycorrhizal fungi and/or dark septate endophytic fungi (Figure 3-4). Additional data on the identity of the fungal taxa would be needed to confirm that claim, and T-RFLP defined taxa are known to be quite coarse in phylogenetic resolution. Subsequent sequence analysis could provide key information to help address this speculation.

It has been suggested that Mer Bleue bog, being in a relatively elevated atmospheric N deposition region, could be P limited (Larmola et al., 2013). As a naturally N-limited ecosystem and past studies suggested P to be the second most limiting nutrient to primary producers in this site, could the increase addition of N alleviated the ecosystem from N limitation and become P limited instead? My data has shown a synergistic role of P and N. This supports the finding by Larmola et al., (2013); microbial respiration was significantly altered in high N plots with the addition of P and K (Table 3-4). The addition of P and K has enhanced microbial community responses to N deposition as shown via T-RFLP and qPCR data. These data were also in agreement with previous studies where P and K seems to enhance ecosystem respiration, microbial biomass (Basiliko et al., 2006), shrub biomass production and peat decomposition (Larmola et al., 2013). Wang et al. (2014) have shown reabsorption of foliar nutrient in shrubs being more responsive to addition of N and PK than N or PK alone. Without knowledge on the

taxonomic composition, particularly for example mycorrhizal fungi, it would be difficult to ascertain if the microbial changes are due to P additions. A follow-up study that includes high throughput amplicon sequencing data is underway, where I will be able to better identify functional groups altered by nutrient additions with higher phylogenetic resolution.

The microbial community changes (Figure 3-7, Figure Figure 3-9) generally corresponded with the vegetation shifts observed in previous studies at Mer Bleue (Bubier et al., 2007; Juutinen et al., 2010; Larmola et al., 2013). Loss of *Sphagnum* moss could be an important factor allowing diversification of microbial communities. *Sphagnum* moss can render conditions inhospitable to other graminoid plants and bryophytes and most mesophilic microbes by sequestering N and leaving the soil nutrient-poor, acidifying through ion exchange, through the production of highly anti-microbial secondary compounds, and because its litter chemical composition is very resistant to decay (Moore and Basiliko, 2006; Rydin and Jeglum, 2013). With higher N loading, the available N concentration is high and allows vascular plants to thrive. The *Sphagnum* moss cover gradually diminishes as incoming vascular plants compete for light through larger foliage. It was also observed in the higher 20N plots that microbial communities are significantly different. The 20N and 20N+PK plots have little to no *Sphagnum* moss left (Larmola et al., 2013).

Loss of *Sphagnum* moss and increases in vascular plants shift the litter type and quality and remove inhibitory properties that might keep microbial activities slow (Larmola et al., 2013). Shrubs and graminoids dominate the higher N and N+PK plots and litters are more labile, permitting rapid microbial mineralization and associated release of nutrients lower in the peat profile (Limpens and Berendse, 2003; Bragazza et al., 2009). Additional nutrients provided by the litter shifts the microbial community as it adapted to new resources (Bragazza et al., 2007).

Data show changes in microbial communities in high N+PK plots (Figure 3-7, Figure Figure 3-10) and in concordance with data from Larmola et al. (2013), there is evidence to suggest that the microbial community shifts occur with the presence of higher litter quality. Microbial diversification potentially provides a broader suite of metabolic capabilities, consistent with findings from substrate utilization assays used by Larmola et al. (2013). However, this speculation requires more in-depth genetic analyses of the soil microbial community. Regardless, changing microbial communities from a *Sphagnum* dominated bog with low decomposition to a shrub and graminoid dominated system with greater microbial diversity can possibly shift the bog's C storing capabilities via altered microbial communities, consistent with very high ecosystem respiration values reported in the same fertilization plots prior (Bubier et al., 2007; Larmola et al., 2013).

Lastly, in 2013, there was a significant impact from N fertilization; as N fertilization increased, microbial communities shifted (Figure 3-7, Figure Figure 3-10). The same pattern was not observed in 2014, despite that the fertilization experiment had run 1 year longer. Increasing soil moisture might have attenuated N effects on fungal communities. Previous studies show that drought and lowered water tables cause increased ecosystem respiration and reduced primary production, creating a net effect of C export (Waddington and Roulet, 1996; Carroll and Crill, 1997; Bubier et al., 2003). A similar pattern was observed in my study sites where ecosystem respiration was higher with lower water table (Bubier et al., 2007). Peatland hydrology has tremendous implications in the biogeochemistry of these systems, specifically at Mer Bleue (Roulet et al., 2007). Data suggest that microbial community dynamic are sensitive to inter-annual environmental fluctuations, in particular water tables, as well as the longer-term effects of nutrient deposition.

3.5 Conclusions and future research

Changes in microbial community composition with high levels of N added could be attributed to vegetation shifts observed by others prior to this study. T-RFLP data provided a broad picture of certain microbial groups (bacteria and fungi) and although it is a generally suitable first step to obtain a rapid fingerprint of microbial communities, ongoing work using sequence analyses of microbial phylogenetic marker genes will cover gaps and provide better information to explore direct and indirect effects of N or P and K additions on the microbial communities.

Microbial communities are adaptable to their environment and my study was perhaps a fair example of this. Moore et al (2011) have observed significant difference in CH_4 fluxes in peatlands between years and water table was the main drivers of those changes. My data suggest high water table positions and soil moisture decreased nutrient impacts in 2014, an abnormally wet year.

In its current state, *Sphagnum* moss at Mer Bleue appears to be actively inhibiting microbial diversity by altering the environmental parameters of the soils and therefore, allowing this ecosystem to sequester C. Changes in litter quality from shifting vegetation and (directly or indirectly) increased nutrient availability are the likely factors shaping an altered microbial community. Increased C quality and nutrients shifts the microbial community and provides altered resources and habitats for new microbes to establish. In light of prior work on microbial functional abilities by Larmola et al. (2013), these new microorganisms appear to bring other functional tools that further decompose the peat soil; decomposition provide more nutrients for plants and a cycle is formed; weakening the C source of peatlands.

3.6 Figures

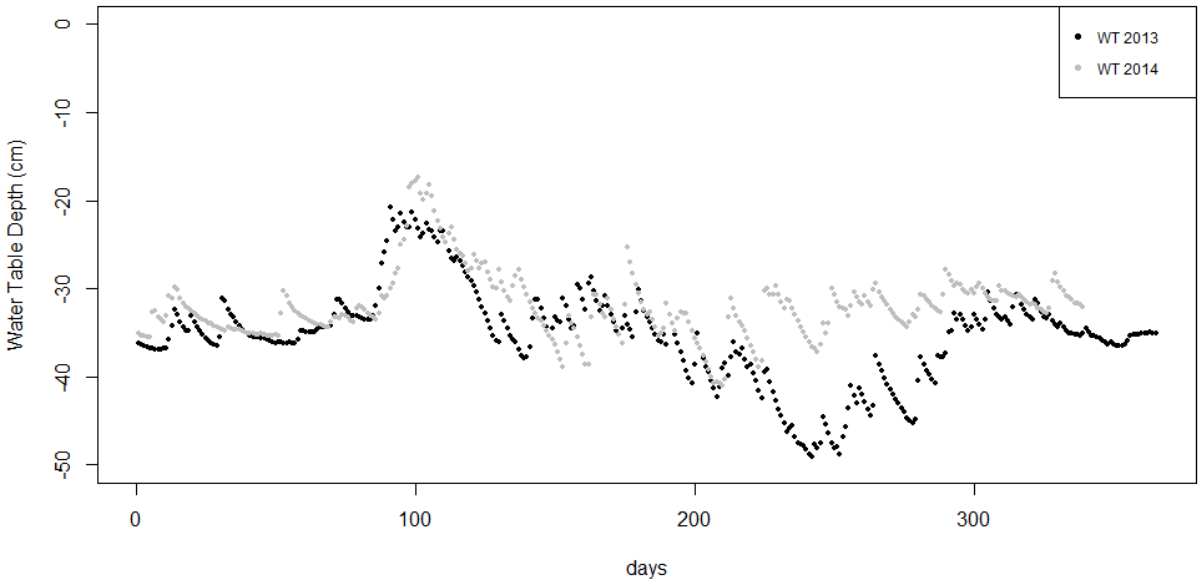

Figure 3-1 - Water table data for 2013 and 2014. Measurements were taken every 30 mins. Daily average water table positions are shown above.

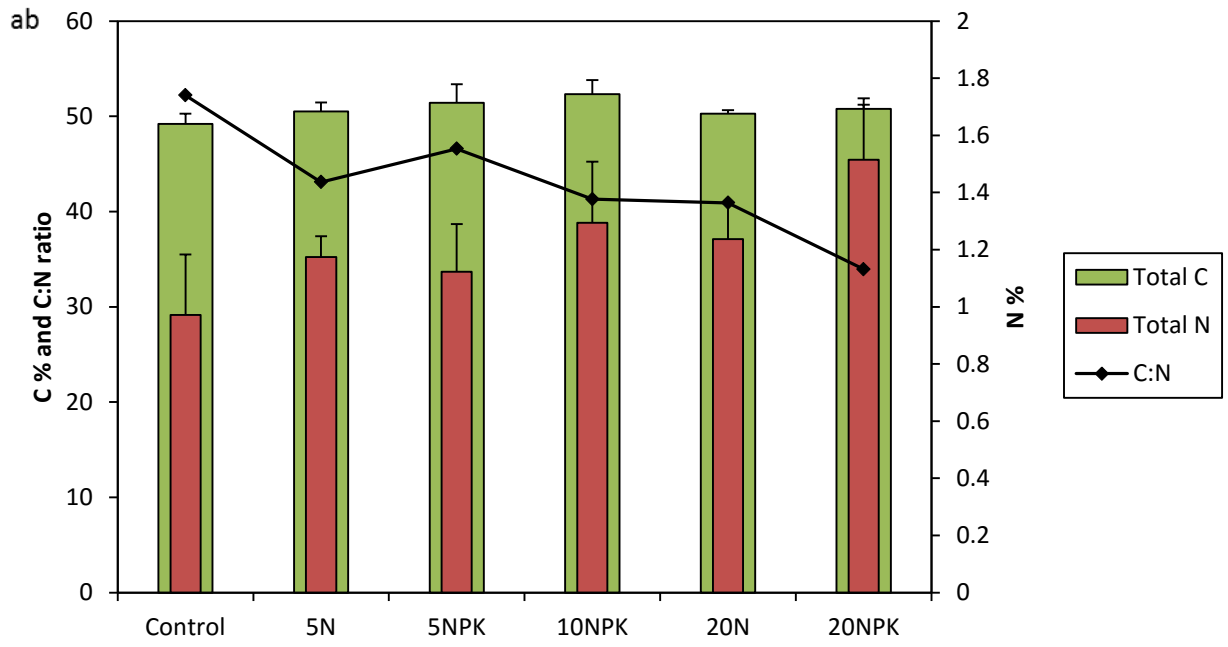

Figure 3-2 - Barplot representing total C and N (%) of bulk soil (mean ± SE). Lineplot represents C/N ratio.

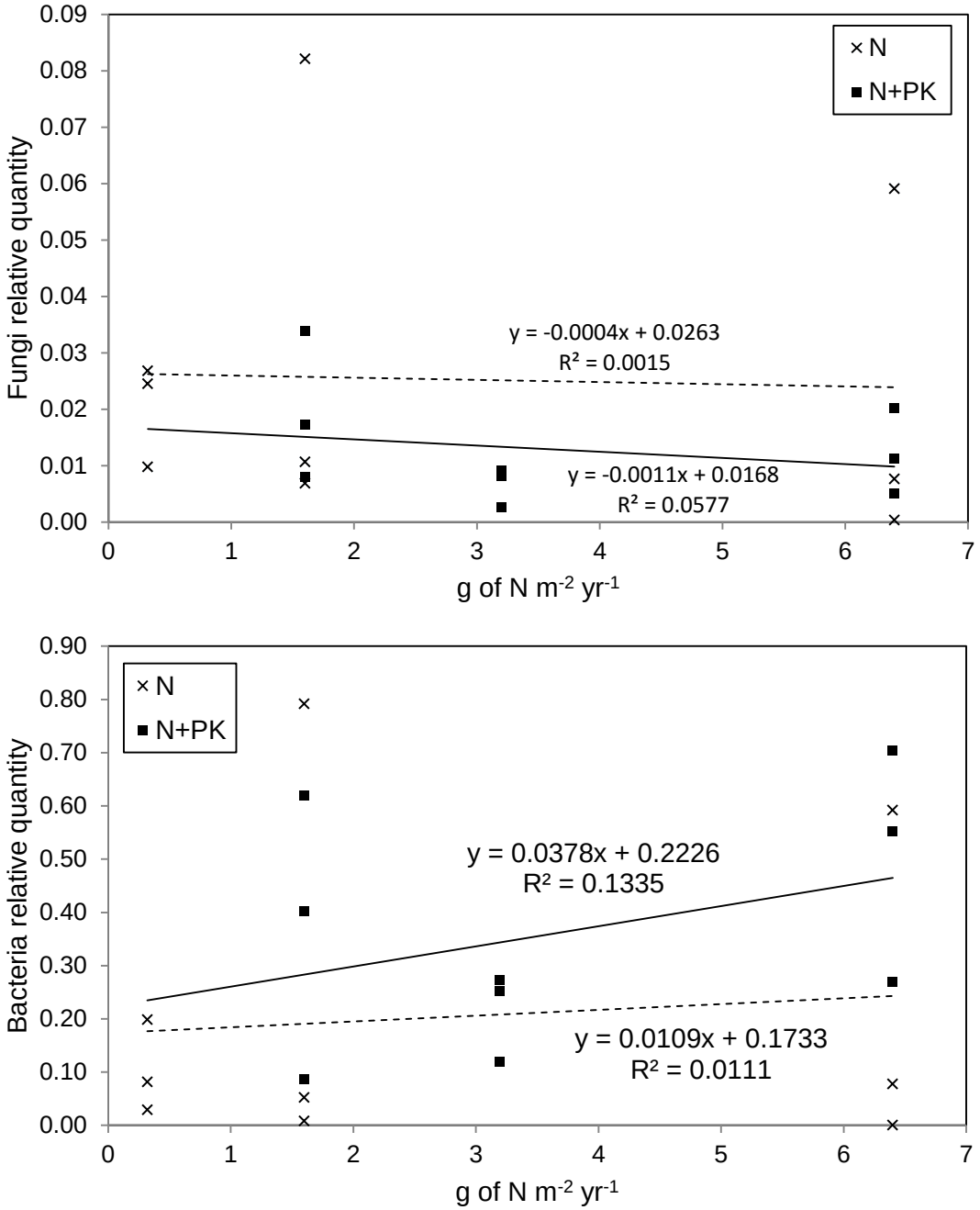

Figure 3-3 - (top) Regression plot of N added vs Fungi 18S rRNA copy number with and without PK of 2013 sampling plots. (bottom) Regression plot of N added with vs Bacteria 16S rRNA copy number and without PK of 2013 sampling plots. (Regression line: N only = dotted; N+PK = solid).

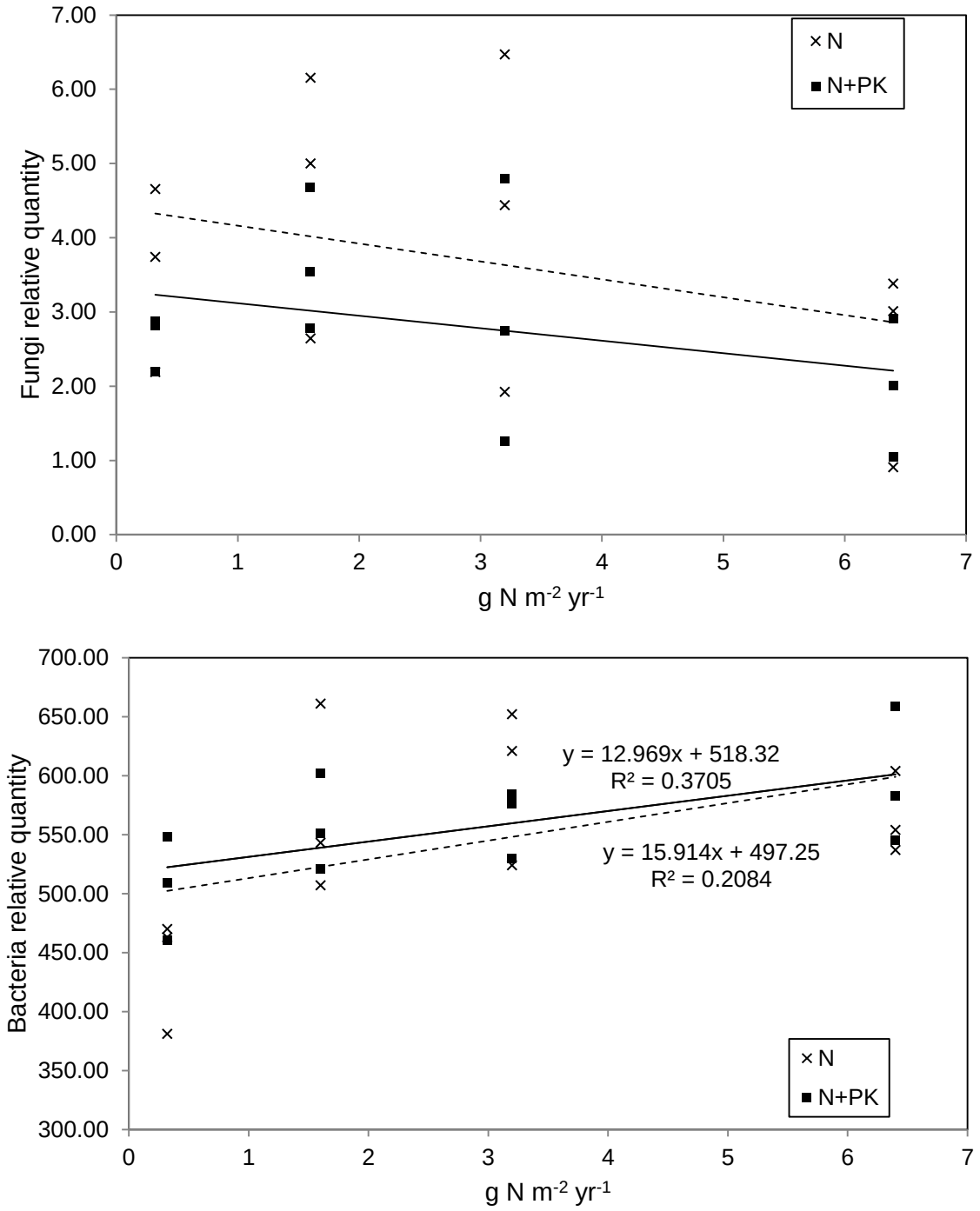

Figure 3-4 - (top) Regression plot of N added vs fungal 18S rRNA gene copy number with and without PK of 2014 sampling plots. (bottom) Regression plot of N added vs bacterial 16S rRNA gene copy number with and without PK of 2014 sampling plots. (Regression line: N only = dotted; N+PK = solid).

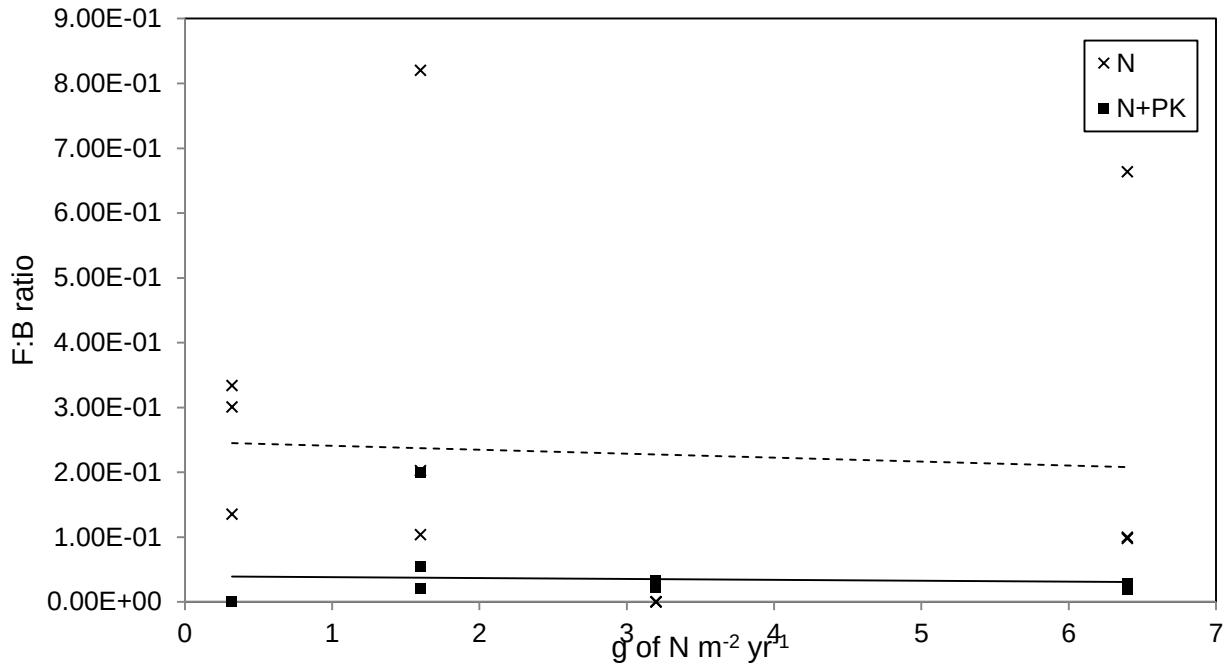

Figure 3-5 - Linear regression plot comparing fungal-bacterial ratios as a function of added N using qPCR data of bacterial 16S and fungal 18S gene copy number in 2013 (Regression line: N only = dotted ; N+PK = solid).

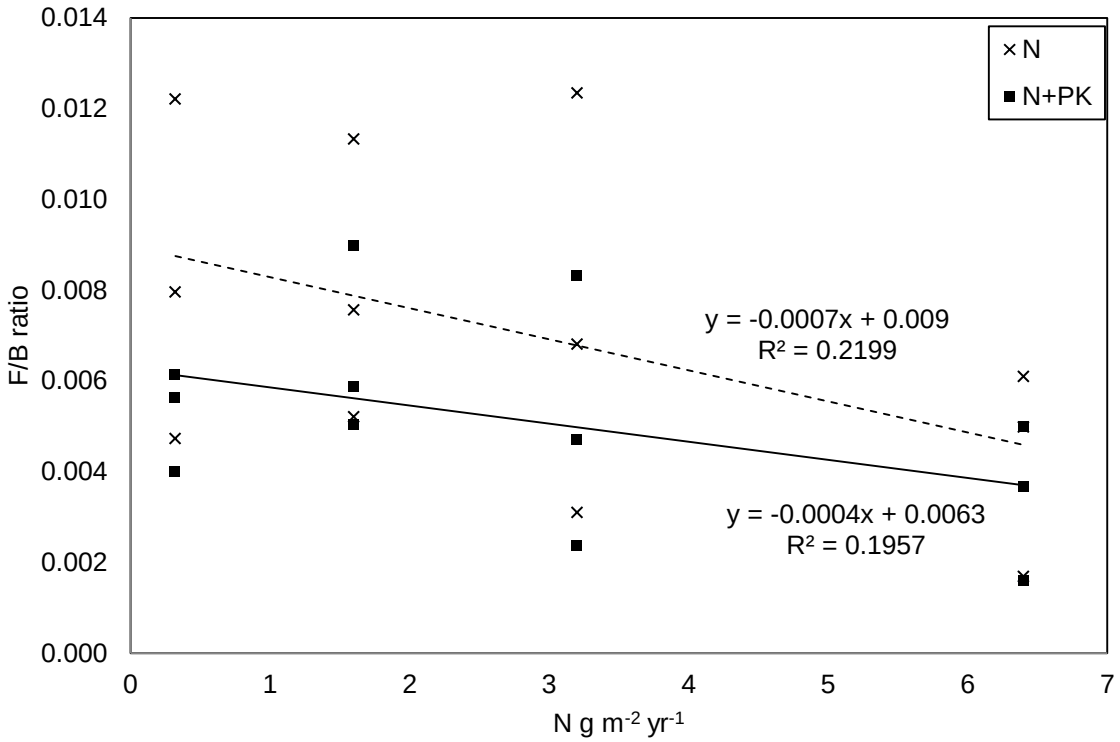

Figure 3-6 - Linear regression plot comparing fungal-bacterial ratios as a function of added N using qPCR data of bacterial 16S and fungal 18S gene copy number in 2014 (Regression line: N only = dotted; N+PK = solid).

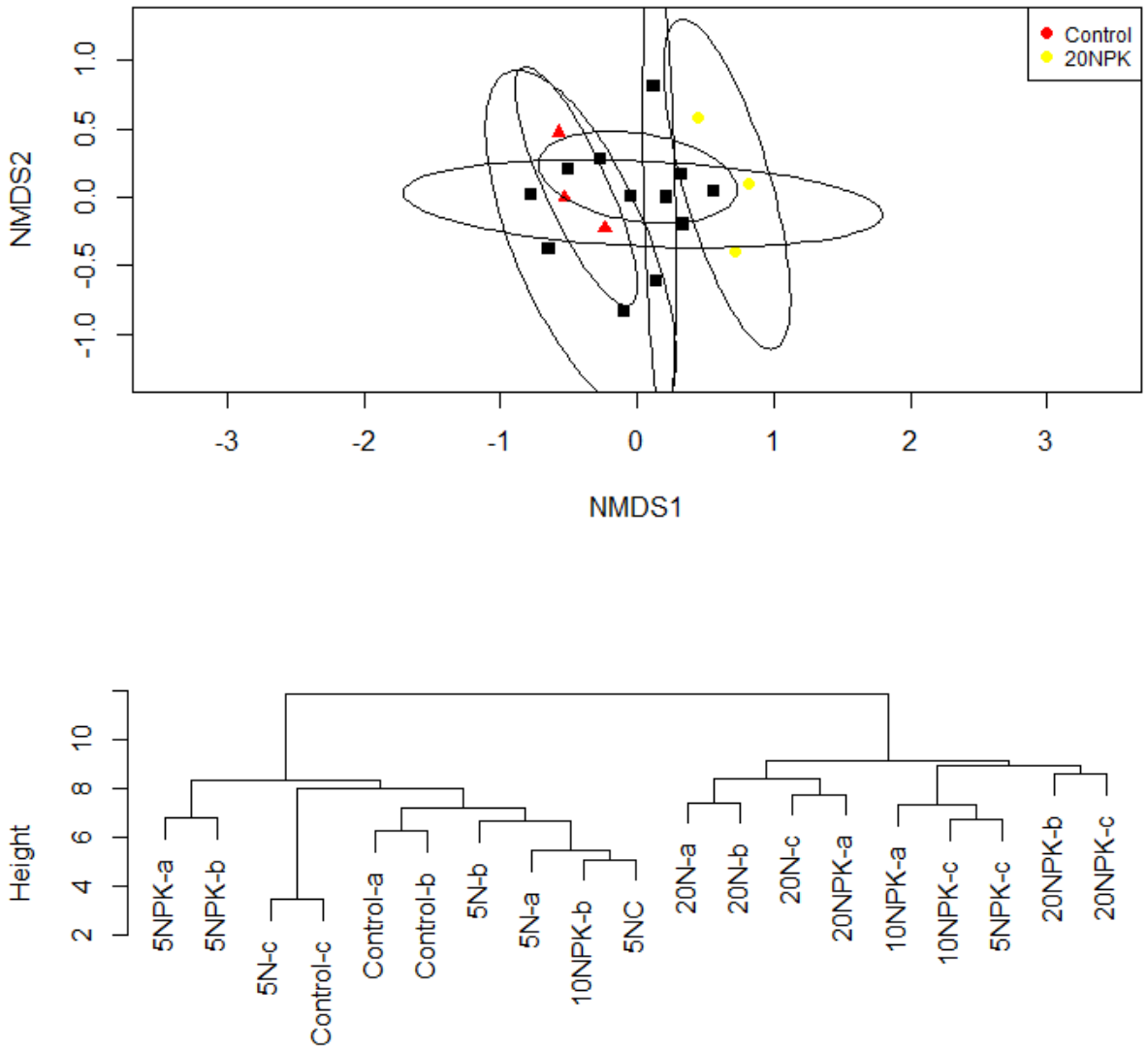

Figure 3-7 - (top) NMDS analysis of bacterial and fungal communities from T-RFLP analysis of 16S and 18S rRNA gens in 2013 (Stress: 0.18). (bottom) Hierarchal clustering of the same data using Ward's methods

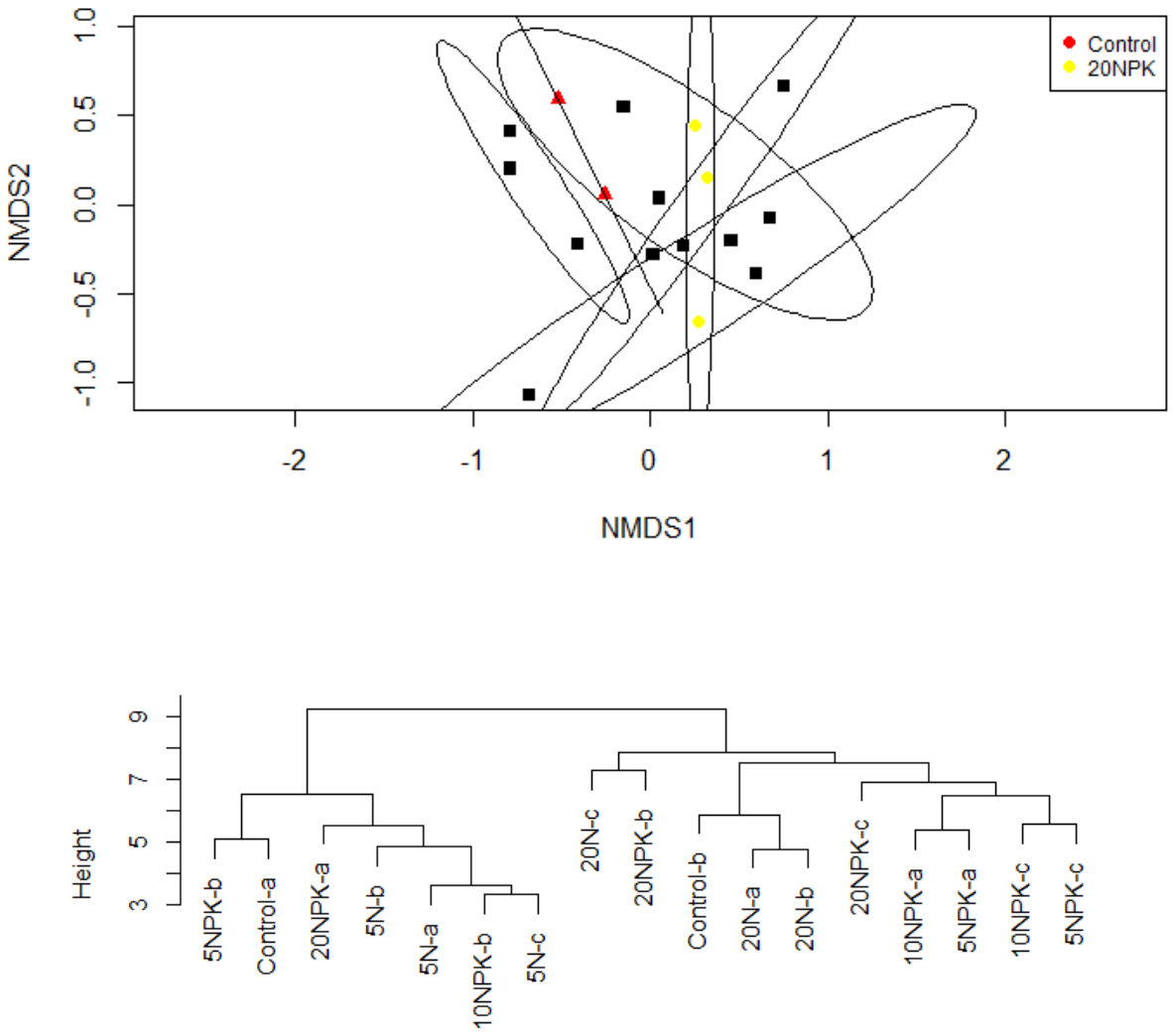

Figure 3-8 - (Top) NMDS ordination of Bacterial 16S rRNA gene T-RFLP communities from 2013 soil samples (Stress = 0.16). (bottom) Hierarchal clustering using Ward's methods of Bacterial 16S rRNA gene T-RFLP communities from 2013 N fertilization samples

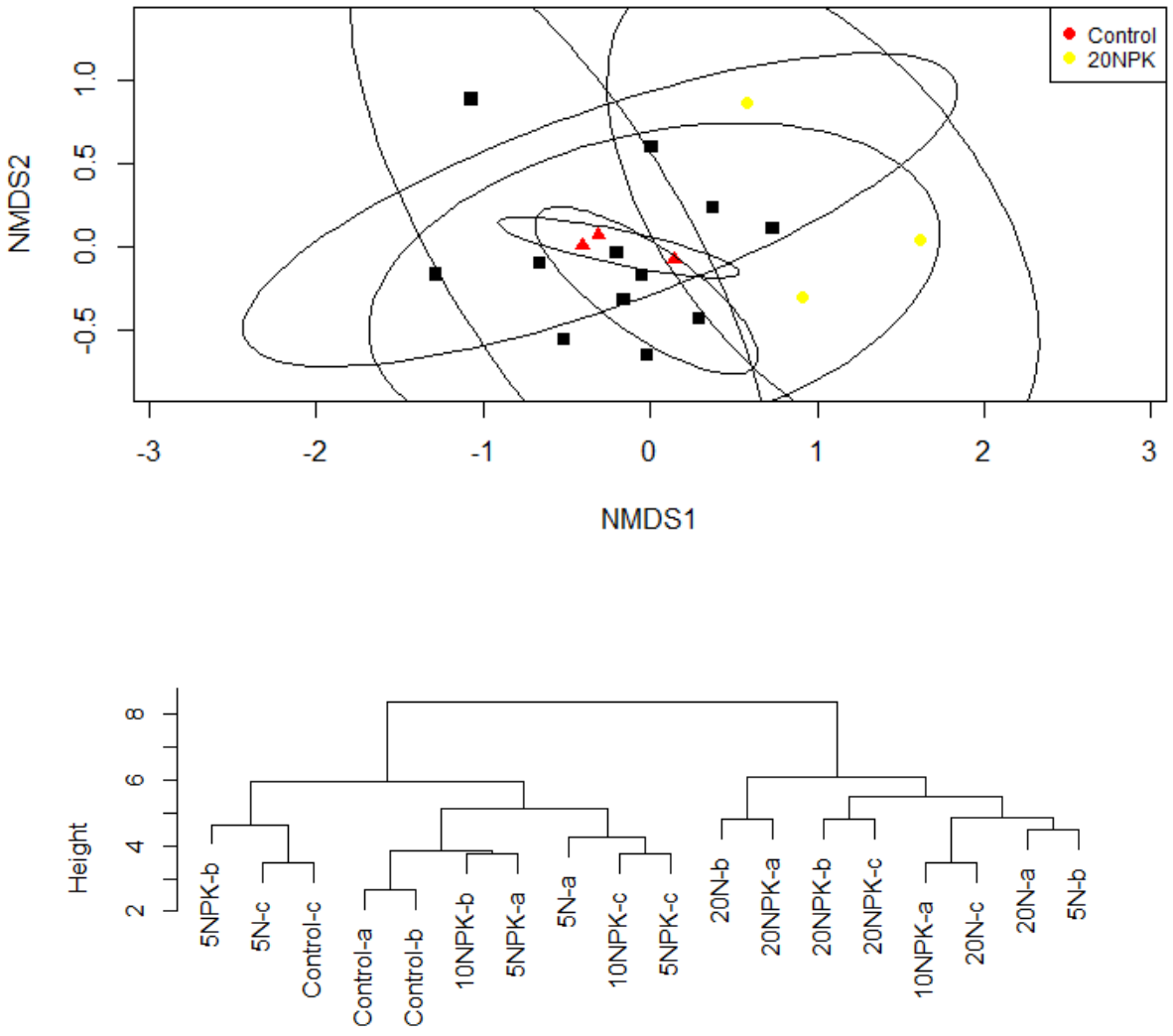

Figure 3-9 - (Top) NMDS ordination of Fungal 18S rRNA gene T-RFLP communities from 2013 soil samples (Stress = 0.14). (bottom) Hierarchal clustering using Ward's methods of Fungal 18S rRNA gene T-RFLP communities from 2013 N fertilization samples.

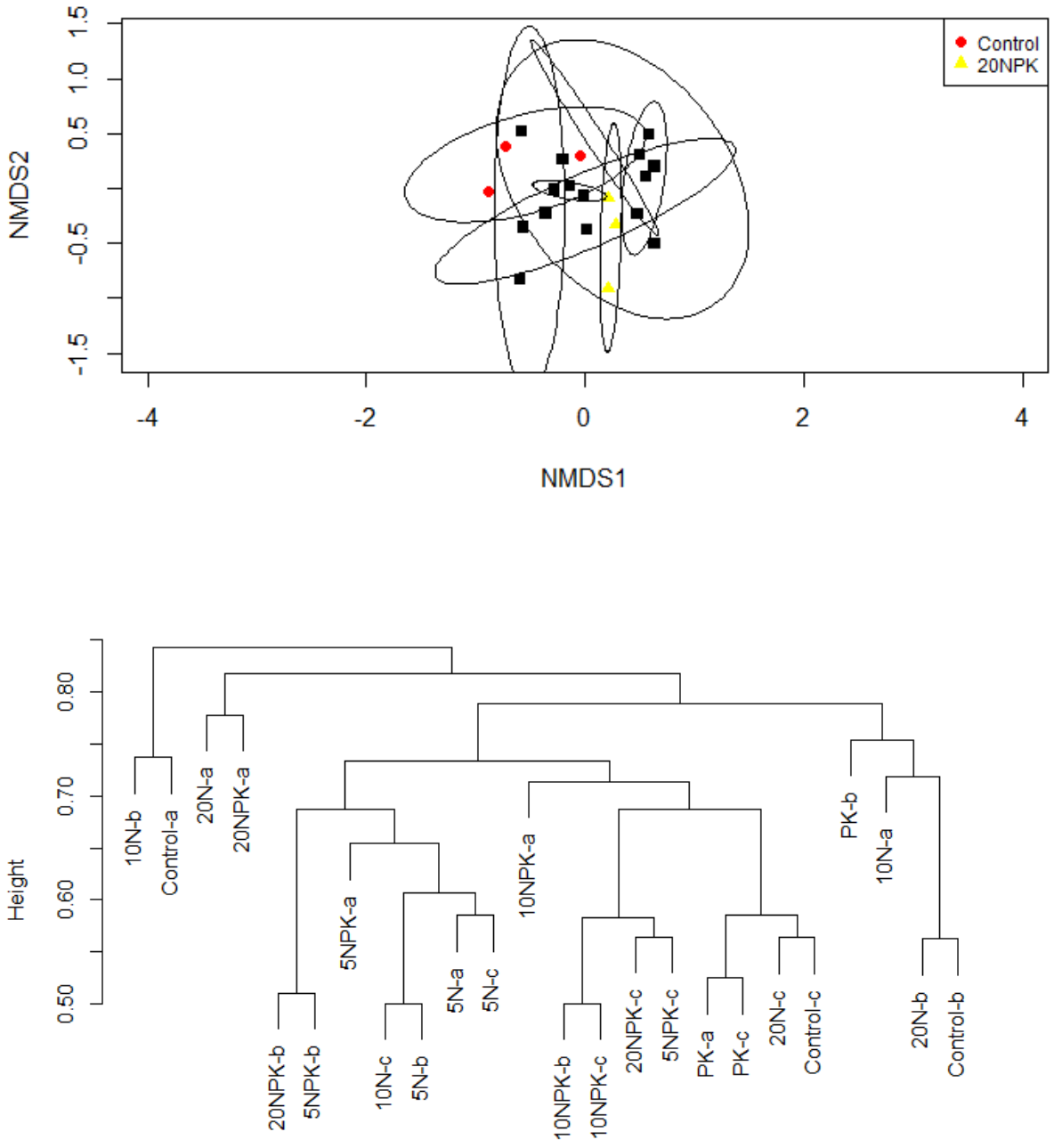

Figure 3-10 - (Top) NMDS ordination of Bacterial 16S and Fungal 18S T-RFLP communities from 2014 soil samples (stress = 0.20). (Bottom) Hierarchal clustering using Ward's methods of Bacterial 16S and Fungal 18S T-RFLP communities from 2014 N fertilization samples

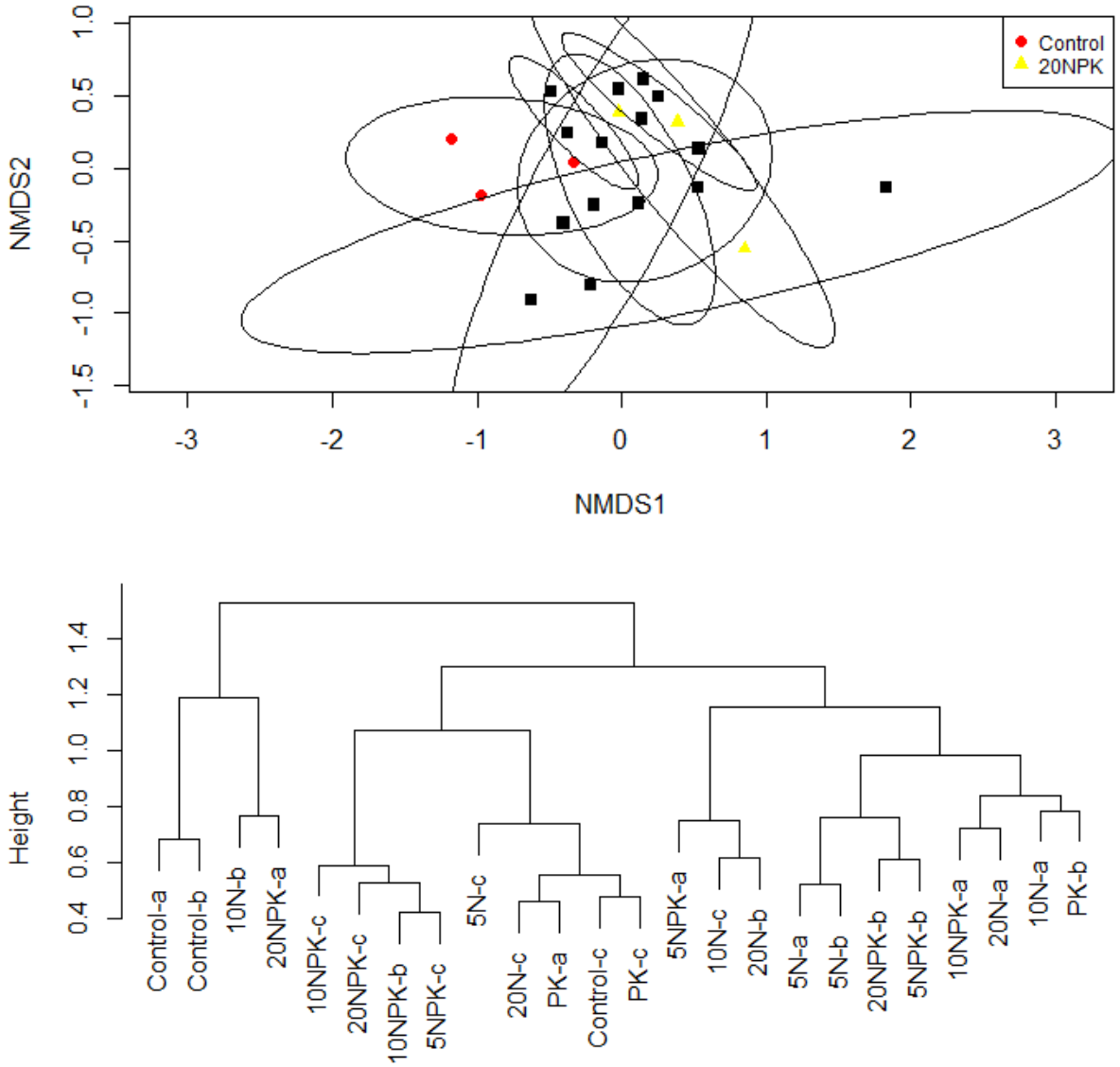

Figure 3-11 - (Top) NMDS ordination of Bacterial 16S T-RFLP communities from 2014 soil samples (stress = 0.18). (Bottom) Hierarchal clustering using Ward's methods of Bacterial 16S T-RFLP communities from 2014 N fertilization samples

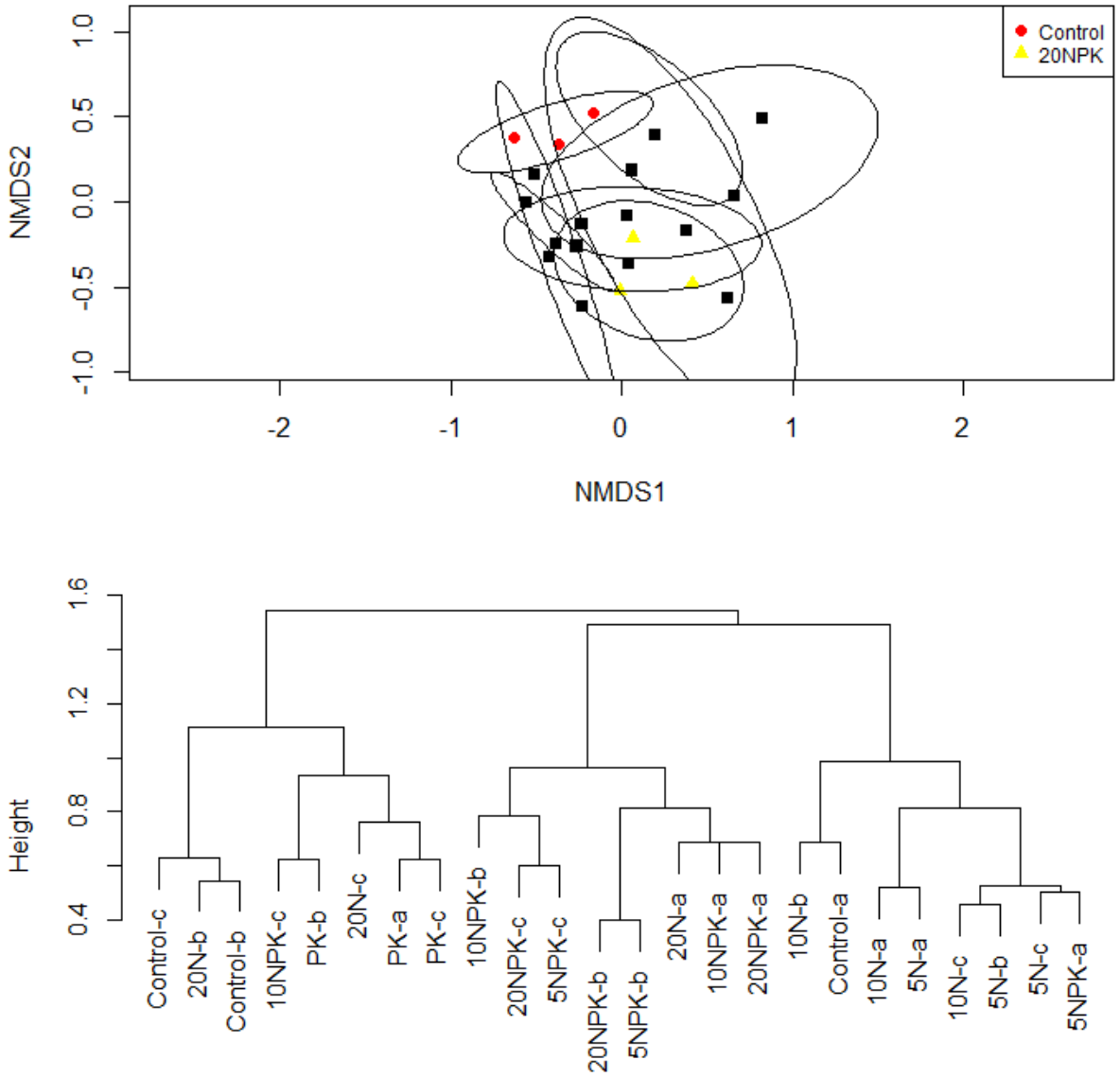

Figure 3-12 - (Top) NMDS ordination of Fungal 18S T-RFLP communities from 2014 soil samples (stress = 0.23). (Bottom) Hierarchal clustering using Ward's methods of Fungal 18S T-RFLP communities from 2014 N fertilization samples

3.7 Tables

Table 3-1 - List of plots sampled in both sampling years

Sample year	***Plots (N only)***	***Plots (N+PK)***
2013	dH_2O (control)	**N/A**
	5N	5N+PK
	N/A	10N+PK
	20N	20N+PK
2014	dH_2O (control)	dH_2O+PK
	5N	5N+PK
	10N	10N+PK
	20N	20N+PK

Table 3-2- Peat soil chemistry as percentage of element by dry weight. Values are mean of percentage composition from triplicate samples. Standard errors are indicated in parentheses. Letters indicate significant differences among treatments (Tukey's test)

	Total N (%)	**Total C (%)**	**P (%)**	**K (%)**
Control	0.97 (0.21)[a]	49.21 (1.06)[a]	0.06 (0.01)[a]	0.12 (0.01)[ab]
5N	1.18 (0.07)[ab]	50.50 (0.96)[a]	0.06 (0.01)[a]	0.12 (0.01) [ab]
5NPK	1.12 (0.17)[ab]	51.40 (1.95)[a]	0.08 (0.04)[a]	0.16 (0.06) [ab]
10NPK	1.30 (0.21)[ab]	52.32 (1.50)[a]	0.09 (0.04)[a]	0.14 (0.08) [ab]
20N	1.24 (0.13)[ab]	50.27 (0.36)[a]	0.05 (0.01)[a]	0.08 (0.01)[a]
20NPK	1.51 (0.21)[b]	50.77 (0.45)[a]	0.10 (0.02)[a]	0.20 (0.02)[b]

Table 3-3 – Shannon and Simpson index of T-RFLP data. Value in each cells indicate average from triplicate plots with standard error indicate in parenthesis.

Treatment plots		***Year***	***Control***	***PK***	***5N***	***5NPK***	***10N***	***10NPK***	***20N***	***20NPK***
Shannon	*Microbial*	*2013*	3.44 (0.54)	**NA**	3.38 (0.02)	3.84 (0.02)	**NA**	3.44 (0.54)	3.77 (0.15)	3.86 (0.20)
		2014	2.93	3.50	3.22	3.15	2.78	3.70	3.37	3.43
	Fungal	2013	2.68 (0.27)	**NA**	2.77 (0.28)	2.89 (0.23)	**NA**	2.59 (0.25)	2.61 (0.46)	2.82 0.16)
		2014	2.50 (0.43)	2.88 (0.14)	2.40 (0.00)	2.71 (0.27)	2.53 (0.12)	2.84 (0.24)	2.70 (0.17)	2.66 (0.40)
	Bacterial	2013	3.30 (0.05)	**NA**	2.55 (0.35)	3.34 (0.15)	**NA**	2.49 (1.55)	3.36 (0.003)	3.39 (0.36)
		2014	2.40 (0.54)	3.24 (0.48)	2.84 (0.39)	2.63 (0.77)	**1.91*** (0.75)	3.46 (0.14)	3.06 (0.44)	3.04 (0.35)
Simpson	*Microbial*	*2013*	0.97 (0.01)	**NA**	0.97 (0.01)	0.98 (0.001)	**NA**	0.96 (0.02)	0.98 (0.003)	0.98 (0.004)
		2014	0.94 (0.02)	0.97 (0.01)	0.96 (0.01)	0.95 (0.02)	0.94 (0.01)	0.98 (0.00)	0.96 (0.01)	0.97 (0.01)
	Fungal	2013	0.93 (0.02)	**NA**	0.94 (0.02)	0.94 (0.01)	**NA**	0.92 (0.02)	0.92 (0.03)	0.94 (0.01)
		2014	0.91 (0.04)	0.94 (0.01)	0.91 (0.00)	0.93 (0.02)	0.92 (0.01)	0.94 (0.01)	0.93 (0.03)	0.93 (0.03)
	Bacterial	2013	0.96 (0.002)	**NA**	0.92 (0.03)	0.96 (0.01)	**NA**	0.81 (0.27)	0.97 (0.003)	0.96 (0.01)
		2014	0.90 (0.06)	0.96 (0.02)	0.94 (0.02)	0.91 (0.06)	0.82 (0.14)	0.97 (0.005)	0.95 (0.02)	0.95 (0.02)

Table 3-4 - Adonis testing results of microbial (combination of bacterial and fungal), bacterial and fungal TRFLP based community data. Significant different ($p < 0.05$) are indicated with an asterisk ($y_{ij} = \mu + N_i + PK_i + \varepsilon_{ij}$).

Sample Year	***Species***	***Treatment (N added)***		***Treatment x PK***		***PK addition***	
	d.f.	*3*		*3*		*1*	
		F	*p*	*F*	*p*	*F*	*p*
2013	*Microbial*	1.19	0.059	1.26	0.10	1.27	0.74
d.f. 12	*Bacterial*	1.30	**0.026***	1.37	**0.05 ***	1.35	0.74
	Fungal	1.16	0.15	1.06	0.34	1.12	0.28
2014	*Microbial*	1.17	0.06	1.51	**0.01***	1.19	**0.04***
d.f. 16	*Bacterial*	1.09	0.23	1.67	**0.01***	1.18	0.09
	Fungal	1.28	**0.02 ***	1.39	**0.04 ***	1.20	**0.05***

Table 3-5 – ANOVA table of linear regression analysis from qPCR data. Asterisks represent data that are statistically significant at $p < 0.05$.

Sample Year		***Treatment (N added)***		***Treatment x PK***		***PK addition***	
d.f.		*3*		*3*		*1*	
		F	*p*	*F*	*p*	*F*	*p*
2013	***F:B***	0.77	0.53	0.025	0.88	4.83	**0.048 ***
d.f. 12	***Fungi***	0.52	0.67	0.017	0.90	0.82	0.38
	Bacteria	0.76	0.54	0.41	0.53	1.42	0.26
2014	***F:B***	1.67	0.21	0.17	0.92	2.54	0.13
d.f. 16	***Fungi***	1.91	0.17	0.097	0.96	2.36	0.14
	Bacteria	5.84	**>0.001 ***	1.12	0.37	0.34	0.57

4. Chapter 2

4.1 Introduction

Peatlands account for 3% of the total land surface but have stored more than one third of the World's soil carbon (C) (Gorham, 1991). Peatlands are generally acidic, waterlogged and cold, which prevents rapid decomposition and renders the soil nutrient limited. Peat soils are depleted in oxygen through some (below water table) or all (complete waterlogged peatland) of the soil profile, resulting in ideal conditions for anaerobic methane (CH_4) production. Considering the land coverage of peatlands, they are important players in the production of atmospheric CH_4, releasing 10% of total emission annually. Anaerobic CH_4 producers or methanogens are coupled with the presence of CH_4 oxidizers or methanotrophs. Most methanotrophs are found in the upper aerobic zones of the soil profile above the water table, but enigmatic anaerobic CH_4 oxidizers might also be important in peatlands (Gupta et al. 2013). Aerobic methanotrophs are important in controlling the amount of CH_4 that is released, and it is believed that 90% of CH_4 produced anaerobically by the methanogens is consumed, thus mitigating net CH_4 emissions substantially (Shannon and White, 1996).

Methanotrophs are ubiquitous gram-negative *Proteobacteria* but are generally concentrated near wet or moist areas where CH_4 is produced. They are unique in their ability to use CH_4 as their soil sources of C and energy (Hanson and Hanson, 1996). They are important players in mitigating CH_4 fluxes in both water/aquatic systems and soils/terrestrial systems. Methanotrophs oxidize CH_4 through a series of biochemical steps; where the first step is the conversion of CH_4 to methanol carried by CH_4 monooxygenases (MMO). There are two different types of MMO: particulate (pMMO) and soluble (sMMO; Murrell et al., 2000). The pMMO operon contains three consecutive open reading frames (*pmoC, pmoA* and *pmoB*) in both type I and type II methanotrophs (that differ in use of serine versus RuMP assimilation pathways;

Stolyar et al., 1999; Gilbert et al., 2000). Most methanotrophs encode the pMMO operon (contains the *pmoA* gene), whereas a restricted few solely encode the sMMO operon (contains numerous genes including *mmoX*), such as the genus of acidophilic, uniquely facultative methanotrophs *Methylocella* found in peatlands (Dedysh et al., 2005). It is suggested that this genus of methanotroph can play a predominant role in CH_4 cycling in acidic *Sphagnum* bogs (Dedysh et al., 2001). *Methylocella* have the ability to utilize other C sources such as acetate, pyruvate, succinate, malate, and ethanol (Dedysh et al., 2005). This relatively recent discovery after methanotrophs had been thought to solely utilize CH_4 for many decades shows that there are likely still many gaps in what is known about methanotrophs' ecology and implications for CH_4 dynamics wetlands.

Bogs are largely anoxic and are home to microorganisms capable of producing CH_4 through anaerobic respiration. These methanogens plays an important role in C cycling in any ecosystem. In fact, methanogens thrive in chemically reduced environment as they are capable of utilizing CO_2 and certain organic electron acceptors allowing them to decompose organic material in anoxic habitats such as wetlands where electron acceptors are limited (Thauer, 1998a). This process involved numerous enzymes and cofactors including methyl-coenzyme M reductase (MCR) coded by the *mcrA* gene (Thauer, 1998b). *mcrA* catalyzes the reduction of methyl-coenzyme M with coenzyme B to CH_4. This gene is unique to methanogens and is considered as a good alternative to the 16S ribosomal RNA gene in phylogenetic analysis of methanogens and other culture independent fingerprinting techniques (Thauer, 1998b; Luthon et al., 2002). It was also reported that this gene could be a good candidate for qPCR analysis (Steinberg and Regan, 2009) and thus could be used to understand nitrogen (N) deposition impacts on methanogen abundance dynamics.

Atmospheric N deposition has increased substantially through fossil fuel combustion and intensive agricultural practices. The excess reactive N has the ability to change the plant communities of bogs as N is often limiting as a plant nutrient. At the Mer Bleue Bog, the effect of N deposition on plant composition is well documented (Bubier et al., 2007). Plant functional types and environmental conditions including soil temperature, precipitation, water table position, and N deposition all can impact both positively and negatively CH_4 fluxes in boreal peatlands (Moore and Knowles, 1989; Larmola et al., 2010; Moore et al., 2011). In fact, a long term fertilization experiment at Mer Bleue indicated a significant shift in vegetation and increased decomposition probably as a results of microbial activities (Larmola et al., 2013). Field studies on CH_4 fluxes were not performed on that site and much remains to be understood about the impact of excess N on CH_4 cycling microbiota in peatlands.

Although it does not support growth, methanotrophs have evolved to be capable of oxidizing NH_4^+ ions into NO_2^-. Both NH_4^+ and NO_2^- have shown to be competitive inhibitor of MMO (Dunfield and Knowles, 1995). In a hypothetical system, where atmospheric N is increased, NH_4^+ deposited could compete with CH_4 for MMO active site leading to CH_4 oxidation inhibition in peatlands (Dunfield and Knowles, 1995; Bodelier and Laanbroek, 2004; Nyerges et al., 2010) potentially leading to an increase in atmospheric CH_4. However, the exact mechanism of N inhibition on methanotrophs is still unknown (Bodelier, 2011).

Other reports indicate that inorganic N addition decreases atmospheric CH_4 emissions through inhibition of methanogens. Competition from nitrate-reducing bacteria for organic C availability and by-products toxic to methanogens could decrease methanogen abundance and activity (Klüber and Conrad, 1998; Fumoto et al., 2007). Decreasing CH_4 production should lead

to a decrease in CH_4 consumption and therefore adding to the assumption that inorganic N addition decrease overall methanotrophy.

New technological advances in molecular biological tools and computational tools can aide immensely in exploring microbial communities. These have provided new insights on methanotrophs in peatlands with high-throughput sequencing technologies (Deng et al., 2013) and quantitative real-time PCR (qPCR) (Dedysh et al., 2005). Genes involved in the oxidation of CH_4 are well-conserved and can make good phylogenetic markers (McDonald et al., 2008). A commonly used functional marker gene is *pmoA*, which encodes the β-subunit of pMMO (belonging to the class of copper-containing membrane-bound monooxygenase) enzymes). A second commonly used functional marker is the *mmoX* gene, which encodes the α subunit of the sMMO enzyme. sMMO is a cytoplasmic enzyme that has a broader range of substrates. Both *pmoA* and *mmoX* have been shown to be congruent with 16S rRNA markers; thus both genes are good candidates as robust phylogenetic markers and for community fingerprinting. Since *pmoA* genes have been sequenced for a larger number of methanotrophs in different environmental settings, and because all known genera of methanotrophs except *Methylocella* contain *pmoA* (Degelmann et al., 2010), it is perhaps the better of the two as a phylogenetic marker tool in sequence-based studies of methanotroph ecology.

The objective of this chapter is to characterize the dynamics and feedbacks of methanotroph communities and CH_4 cycling activities to N fertilization at the Mer Bleue Bog near Ottawa, Canada. I used physiological incubations to observe CH_4 oxidation and production potentials from soils collected across the experimental plots. To understand inorganic N addition on methanotrophy, I characterized methanotroph community structure using high throughput amplicon sequencing of the *pmoA* gene and qPCR of *pmoA* and *mmoX* genes. Additionally, to

quantify potential changes in methanogen abundances, I also characterized *mcrA* gene copy number using qPCR.

Based on previous studies, I hypothesize that methanotroph communities will change as a results of an increase inorganic N addition. As NH_4+ has been documented to inhibit the CH_4 consumption pathway, it could have detrimental effect on methanotrophs diversity and abundance. Moreover, reports have indicated dramatic changes in vegetation and plant community when comparing control and low N to higher N plots. Such dramatic changes in this ecosystem should also have an impact on microbial community including the methanotrophs. Furthermore, it was reported that nitrite is potentially toxic to methanogens, the addition of inorganic N on the fertilization plots could cause deleterious effect on methanogens abundance.

4.2 Methods

4.2.1 Experimental site and sampling

The Mer Bleue bog (45°24' N latitude, 75°31' W longitude), located 10 km east of Ottawa, Canada, is a large raised ombrotrophic peat bog complex with hummock-hollow patterned topography. It is *Sphagnum* moss dominated with an evergreen shrub overstory. Background N deposition is among the highest in Canada with an approximate annual deposition of 1.5 N g^{-2} yr^{-1}. Regional total N deposition rate was estimated to be 0.6 to 0.8 g N m^2 yr^{-1} (Keith and Dillon, 1989; Sisterson et al., 1994). The water table depth typically averages 30 cm beneath the surface of the hummocks and in the 2014 growing season when I collected samples it was -32.0 cm.

CH_4 oxidizing bacteria are assumed to be found in soil depths above the oxic- anoxic interface or in close proximity to oxygen conducting plants issue within the anoxic layer. This

interface allows both the presence of CH_4 and oxygen (Henckel et al., 2000). For that reason, samples at 30-40 cm depths were used to study the impact of N and/or P and K on methanotrophs. On the other hand, methanogenesis is a strictly anaerobic heterotrophic process utilizing a select range of small organic molecules or H_2 and CO_2 (Freitag and Prosser, 2009). Therefore sampling at the depth below the oxic-anoxic layer (50-60 cm depth) should target active methanogenic communities, as they are in a zone of anoxia, but still relatively "fresh" peat and C and redox substrates, versus in more recalcitrant organic matter at lower depths. For my experiments, 30 – 40 cm depth samples were used for studying methanotroph communities, while the 50 – 60 cm was used for methanogen communities.

4.2.2 Field Fertilization

Field fertilization experiments were established in 2000-2001 (Bubier et al., 2007). Fertilization plots (9 m^2 in area each; 3 replicates per treatment) were set up in a 400 m^2 section of the bog with little variation in micro-topography and vegetation. Solutions of N were added every three weeks as ammonium nitrate (NH_4NO_3) at 1.6**,** 3.2 and 6.4 g N m^{-2} yr^{-1}, approximately 5, 10 and 20 times the ambient growing season atmospheric deposition. Phosphorus and K were added as potassium dihydrogen phosphate (KH_2PO_4), equivalent to 6.3 g K and 5.0 g P m^{-2} yr^{-1}. Control plots received P and K solution or distilled H_2O. Plots were identified as Control, PK, 5N, 5N+PK, 10N, 10N+PK, 20N and 20N+PK. Treatment plots were relatively large, close to each other and located in a homogenous area of vegetation and topography. Also, large concentration of nutrients was added to those plots. As a result, I assumed that any consistent observed difference across replicates were due to fertilization treatments.

For each plot, three cores were taken in 2014 using a Russian corer that was thoroughly washed between samples. Soil depths of 30-40 and 50-60 cm below the vegetation layer representing the horizons above and below the water table were sampled from each core and places in sterile plastic bags, combining three cores into a single bag. Each bag was homogenized by mixing the sample mechanically by hand. Sample bags were placed in a cooler with ice until they were transported to Laurentian University where they were stored at 4°C.

4.2.3 CH_4 oxidation

Mason© glass jars of 250 ml volume were used as microcosms for measuring soil CH_4 oxidation potential. Twenty grams of moist peat from each sample was put in a jar sealed with a modified metal lid with an incorporated butyl rubber septum. For each sample, pure CH_4 was added at 1% (v/v) CH_4 (resulting in a 10 000 ppm CH_4 headspace concentration) and incubated in the dark at room temperature for a two days. 10 ml of headspace air was sampled at 0, 6, 12, 18, 24 hours post-CH_4 addition. To maintain pressure within the jar, 10 ml of room air was injected into the jar. Jars were shaken prior to sampling. Syringes were injected into a SRI 8100C gas chromatograph (Torrence, CA, USA) with a flame ionization detector. After the incubation period, jars were weight prior- and post-drying where they were placed in an oven at 70°C overnight. Dry peat mass and headspace volume was also measured. Chromatograph peak data were converted to µg CH_4 g^{-1} dry peat and µg CO_2 g^{-1} dry peat based on known standards and using the ideal gas law. CH_4 oxidation potentials were calculated by linear regression of µg CH_4 g^{-1} dry peat as a function of time. CO_2 production potential was calculated by linear regression of µg CO_2 g^{-1} dry peat as a function of time. Cumulative CH_4 oxidation potentials and CO_2 production potentials were corrected for loss of mass of gasses during multiple samplings.

4.2.4 CH_4 production potential

Conical centrifuge tubes of 50 ml volume (BD Falcon, Bedford, MA, USA) were used as a mesocosm for measuring soil CH_4 production potential. For each sample 10 g of moist peat (50-60 cm) was placed in the tubes and sealed with modified plastic caps with incorporated butyl rubber septa under anoxic conditions in a glove box. For each sample, tubes were incubated in a controlled incubator at 25°C for 80 days. 5 ml of headspace air was sampled at 0, 40 and 80 days post-incubation. To maintain negative pressure within the jar, 5 ml of N_2 was injected into the jar. Jars were shaken prior to sampling. Syringes were injected into a SRI 8100C gas chromatograph (Torrence, CA, USA) with a flame ionization detector. After the incubation period, jars were weight prior- and post-drying where they were placed in an oven at 70°C overnight. Dry peat mass and headspace volume was also measured. Chromatograph peak data was converted to µg CH_4 g^{-1} dry peat based on known standards and using the ideal gas law. CH_4 production potential was calculated by linear regression of µg CH_4 g^{-1} dry peat as a function of time. Cumulative CH_4 production potentials were corrected for CH_4 loss during multiple samplings.

4.2.5 DNA extraction

DNA was extracted from wet peat samples as per the method indicated in the PowerSoil® DNA Isolation kit (MO BIO Laboratories Inc., Carlsbad, CA). All DNA samples used in subsequent experiments were pooled from three extractions with total DNA concentrations of 25 to 50 ug/uL. Peat disruption and homogenization was performed using a 5 minute cycle on a 16 tube MiniBeadbeater TM (Biospec Products Inc., Bartlesville, OK). DNA samples were stored at -20°C for analysis at a later date.

4.2.6 Pyrotag sequencing

Pyrotag sequencing of *pmoA* gene fragments on the Roche 454 platform (at Molecular Research LLP) was completed on samples from the 30 to 40 cm depth horizon per Dowd et al. (2008). Forward primer A189 and reverse primer mb661 (Table 4-1) were used and amplified an approximately 470-bp internal section of *pmoA* as described by Costello et al. (1999). All replicated plots were treated as individual samples.

4.2.7 qPCR

The *pmoA* and *mmoX* functional genes were qPCR-amplified using oligonucleotide primers and following methods described in Costello and Lidstrom (1999) and Rahman et al. (2011) respectively. Primer sequences are listed in Table 4-1. qPCR assays were conducted in polypropylene 96–well plates on an Agilent Technologies Stratagene MX3005P qPCR system. Each 20 µl reaction contained the following: 10 µl of Thermo Scientific DyNAmo HS SYBR green qPCR 2X Master Mix, 0.5 µl of each primer sets 9 µl of dH_2O. All PCR have a heating step of 15 mins at 95°C. PCR conditions for *pmoA* were 45 cycles of 95°C for 30 sec, a two-step annealing at 56°C for 30 sec and 60°C for 30 sec, and extension at 77°C for 30 sec. Data were collected at the end of extension phase. As for *mmoX*, 45 cycles of melting at 95°C for 15 sec and an annealing at 68°C with no extension. Data were collected at the end of annealing phase. Finally, PCR conditions for *mcrA* were 45 cycles of melting at 95°C for 15 sec and an annealing at 68°C with no extension. Data were collected at the end of annealing phase. All reactions, including sets of standards, were performed in triplicate. After each qPCR run, melting curve analysis was performed to verify the presence of the desired amplicon and to confirm products were not from primer-dimers or other artifacts. Three dilutions of known concentrations of

Methylococcus capsulatus (Foster and David) ATCC ® 19069 ™ and *Methylocella silvestris* (Dunfield) DSMZ 15510 DNA were used as standards for *pmoA* and *mmoX* quantification respectively.

To evaluate the gene copy abundance with the 50 – 60 cm anoxic zone of my soil samples, the *mcrA* gene was qPCR-amplified using oligonucleotide primers that target a broad range of methanogens following methods described in Steinberg and Regan (2008). Primers are listed in (Table 4-1). PCR conditions for *mcrA* were 15 min at 95°C, followed by 45 cycles of melting at 95°C for 30 sec, annealing at 50°C for 30 sec and extension at 72°C for 30 sec. Data were collected at the end of annealing phase. Dilutions of known concentrations of *Methanoregula boonei* 6A8 (Brauer et al., 2010) DNA were used as standards for quantifying *mcrA* genes.

4.2.8 Statistical analyses

Gas analyses results were analysed using regression analysis using standard R packages. Regression analysis was performed to determine the effect of N additions on activity measurements.

Standard curves were produced using triplicate 10-fold dilutions of DNA from pure culture *M. capsulatus* for *pmoA* and *M. silvestris* for *mmoX*. At least three nonzero standard concentrations per assay were used with concentration ranging from 10^{-2} to 10 ng of DNA per reaction. Target copy numbers were calculated from the standard curves using threshold cycle value (CT). For all qPCR assays, there was a linear relationship between the log of the DNA concentration and the calculated threshold cycle value (CT) across the specified concentration range ($R^2 > 0.95$). Linear regression analyses were performed on N deposition and with and without P and K as an effect to relative gene copy number.

Molecular amplicon sequence datasets were analysed using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (Caporaso et al., 2010). Data were quality filtered using QIIME default parameters (quality score = 25, min length=200, max length = 1000). Additional quality filtering and operational taxonomic unit (OTU) clustering was performed with the Usearch 5.2.236 program, which utilizes the UCHIME algorithm to identify chimera sequences for removal against the *pmoA* methanotroph dataset from Dumont et al. (Edgar, 2010; Edgar et al., 2011; Dumont et al., 2014). De novo OTU picking with uclust was used to form the representative OTU dataset (Edgar, 2010). Bacterial taxonomy was assigned using the RDP classification algorithm against the *pmoA* dataset of Dumont et al. (2014). MUSCLE was used to produce a denovo alignment of all OTU sequences and produce a neighbour-joining phylogenetic tree (Edgar, 2004). 48 Final OTUs represented potential species based on unique *pmoA* sequences that were discriminated at a level of 97% similarity.

Subsequent community structure analyses were conducted in the QIIME pipeline and in the R phyloseq package (McMurdie and Holmes, 2013; R Core Team, 2013; Oksanen et al., 2014). Microbial communities between samples were compared using NMDS (Bray-Curtis distance) with the vegan package. OTUs were analyzed in the context of relative abundance. Using QIIME, area chart plots were generated to observed relative taxa abundances across my samples and treatments. An agglomerative hierarchical cluster analysis based on Ward's method of dissimilarity calculation was used to provide another indication of the relatedness among the treatments. Adonis tests were used to determine if communities of different treatments were significantly different from each other.

4.3 Results

4.3.1 CH_4 production and oxidation

At the 30 – 40 cm depth, I observed no significant effects of N and/or PK additions on methanotrophy ($p = 0.35$; Figure 4-1); oxidation rates were low and variability among replicates was high (Figure 4-1). In some instances, I observed slight CH_4 production (control = 3.83 μg g^{-1} dry peat, 10N = 4.06 μg g^{-1} dry peat, 10N+PK = 0.81 μg g^{-1} dry peat, 20N+PK 1.91 μg g^{-1} dry peat). At the 50 – 60 cm depth, anaerobic CH_4 production also showed no significant changes with N addition ($p = 0.60$; Figure 4-2). Gas production rates among all plots were small. Addition of P and K also had no statistically significant effect on CH_4 production rates. However, I observed a weak, non-significant decreasing trend in CH_4 production with both N only addition and N with addition of P and K plots.

4.3.2 Methanotroph communities

A total of 83 161 *pmoA* reads across the 27 samples/experimental plots were generated with "Usearch" OTU picking parameters identified 1 206 distinct OTUs. More than 50% were unmatched sequence to the BLAST database and, upon further investigation; they were deemed as sequencer or computational artifacts and were discarded from further analyses.

Using adonis tests, no significant (d.f. = 8, $p = 0.16$) *pmoA*-based community differences in methanotroph communities among the fertilization treatments were observed (Figure 4-8, Figure 4-9). NMDS showed 95% confidence interval ellipses that are overlapping by treatments and controls, meaning N addition or P and K addition had no significant impact on (*pmoA*-containing) methanotroph communities. Similarly, cluster analysis of plots also demonstrated no observed patterns by treatment; thus no effects can be attributed to additions of N and/or P and K (Figure 4-8). Moreover, phylogenetic identification across all plots illustrated that most methanotrophs fell within a few select genera (Figure 4-7); with *Methylocystis sp* and *Methylomonas sp* representing the majority of the *pmoA*-containing methanotrophs and with

Methylocystis dominating across all plots. A stacked area chart reveals the coarse-scale phylogenetic assemblages of methanotroph communities among all treatment plots; there were no clear effects of N and/or PK loading on methanotroph communities (Figure 4-6).

4.3.3 Functional gene abundance

My data indicated no significant changes in *mmoX* (d.f. = 3; p = 0.55) or *pmoA* (d.f. = 3; p = 0.24) copy number from the N and/or P and K fertilization (Figure 4-3, Figure 6-8). However, the CH_4 production marker gene *mcrA* copy number significantly decreased with increasing N addition (P =0.021, Figure 4-5). Addition of P and K (alone or with N) had no effect on *mcrA* copy number. I found that there were no significant changes in the ratios of *mmoX* to *pmoA* genes, with increased N addition.

4.4 Discussion

My goal was to investigate the potential effects of N addition on methanotrophs and methanogens. Both are key microorganisms in the net emission of CH_4. I have shown that with the addition of nutrients, the methanotroph communities did not change significantly or increase in numbers (Figure 4-1, Figure 4-3, Figure 4-4, Figure 4-9). As added N and nutrients having been assimilated or immobilized in the upper profile, it might not have reached lower depths such as the 30 – 40 cm used in my experiment (Basiliko et al., 2006). Inorganic N would not have the opportunity to disrupt methanotrophy or methanogenesis directly. I have hypothesize that methanogen abundance would decrease as a results on nitrite toxicity from denitrifiers. It would not be possible as samples taken for the study of methanogen abundance are of 50 – 60 cm depth. If N and nutrients added are rapidly consumed at higher level of the soil profiles, N would not have reached that depth.

In situ CH_4 emissions and microbial production and consumption processes are closely linked to water table levels. As water tables rise, one usually observes an increase in CH_4 production and emissions (Moore et al., 2011). In 2014, water table measured at Mer Bleue was high meaning higher soil moisture (Figure 3-1). While, I should have observed an increase in CH_4 production potential, my data indicate no significant changes. Since my experiment was performed in mesocosm, it might not reflect the situation *in situ* as many environmental factors might have played a role in increasing CH_4 production. Higher water table position in higher N plots is due to a depression of the peat layer that is at large decomposing more quickly than control plots (Larmola et al., 2013), bringing the vegetation layer closer to the water table; the roots and rhizosphere are also closer. Moreover, past studies showed that belowground plant biomass was also higher with depth (Murphy and Moore, 2010). If one considers the argument that the new, highly abundant vascular plants are providing oxygen via root systems to the peat profile. Also, sedges, such as *Eriophorum vaginatum* have increased greatly with fertilization could facilitate the transfer of oxygen from aboveground to the rhizosphere through aerenchyma in their roots (Murphy and Moore, 2010). Methanogens are strict anaerobic microorganisms, Exposure of anaerobes to oxygen results in autoxidation of cellular components causing oxidative damage, and may ultimately lead to cell death (Imlay, 2002). It would seem that N deposition is indirectly affecting methanogens via the vegetation shift. Following the same logic, as the methanogen abundance decreases, CH_4 concentration at the oxic-anoxic zone would be lower. However interestingly this did not lead to changes in methanotroph abundance or to CH_4 oxidation rates.

Surprisingly, despite changes in vegetation cover and broad-scale microbial communities reported earlier in the Mer Bleue fertilization experiments (Basiliko et al., 2006; Larmola et al.,

2013), my data indicate no changes in CH_4 oxidation potential as addition of N increases (Figure 4-1, Figure 4-3, Figure 4-4). However this finding is at least consistent with the lack of change in CH_4-oxidizing bacterial community structure. Application of inorganic N has been documented as detrimental to atmospheric CH_4 uptake by soils (Bodelier and Laanbroek, 2004). In my fertilization experiments, inorganic N is deposited, potentially providing enough ammonium substrate to compete with CH_4 for MMO active sites (Nyerges and Stein, 2009). As well, osmotic effects due to salt additions and inhibition by nitrite, which is toxic to methanotrophs, were also potentially expected, but not observed. Another potential explanation for the lack of observed patterns would be that the inorganic N was quickly consumed by overlaying organisms (vascular plants and microbes) and has not reached the 30-40 cm depth. Additionally, CH_4 production potential data suggest no increase input in CH_4 in the same layer, therefore not stimulating methanotrophs community. However, my data does not provide any indication of stimulating methanotrophy as there was no evidence of increase CH_4 production. Observing methanotrophs along the soil profile at various depth could provide insight on the direct effect of increasing N on methanotroph community and abundance.

Members of the genera *Methylomonas* and *Methylocystis* dominated the methanotroph communities regardless of treatments (Figure 4-6, Figure 4-7). This is similar to a pattern seen in a Dutch peat bog (Kip et al., 2011). *Methylocystis sp*. are commonly found in ecosystems where the soil is acid ($pH < 5$) and CH_4 is present in high concentrations (Dedysh et al., 2007; Belova et al., 2013). *Methylomonas* have also been found in other bogs (Chen et al., 2008; Kip et al., 2011). *Methylocystis* and *Methylomonas* have both MMO genes (*mmoX* and *pmoA*), and their predominance could explain why *mmoX* (Figure 4-3) and *pmoA* (Figure 4-4) qPCR data remained unchanged across treatments.

Many factors could explain *Methylocystis* dominance in my plots. Mer Bleue bog pore water was acidic (pH = 4.3). Another factor, would be the newly discovered versatility of *Methylocystis* in multi-carbon compounds metabolism capabilities. Belova et al. (2013) demonstrated *Methylocystis sps.* can utilize other small organic molecules including acetate when CH_4 is limited. This strategy could be a reason for its dominance in my soils, if CH_4 concentrations are low.

4.5 Conclusion

Contrary to my initial hypotheses, my data indicated no changes in methanotrophy, concurrent with an unchanged methanotroph community after prolonged increased N and /or P and K deposition; consequently the net effect of N fertilization on *in situ* CH_4 dynamics might be dictated by the nutrient effects on CH_4 production. My data show CH_4 production potential in controlled experiment has not changed significantly but that *mcrA* gene copy numbers were significantly reduced with N addition. Past experiments also showed a decrease in CH_4 production *in vitro,* however, field CH_4 fluxes data at Mer Bleue indicate increasing CH_4 efflux as N deposition increased (Armés, 2009). CH_4 dynamics seemed to be the result of a much more complex system where additional environmental data are needed to fully understand inconsistencies between *in vitro* approaches here and *in situ* gas fluxes. Moreover, more comprehensive understanding of how inorganic N potentially impacts methanotrophy and methanogenesis could be achieved through more controlled (e.g. lab-based) peat fertilization experiments. . While using next-generation sequencing does provide useful information on community structure and phylogenetic identity of organisms, further work isolating key organisms in culture, followed by specific tests of nutrient loading on isolates (and resulting

physiological changes) could also better help us understand the dynamics involved in CH_4 cycling.

4.6 Figures

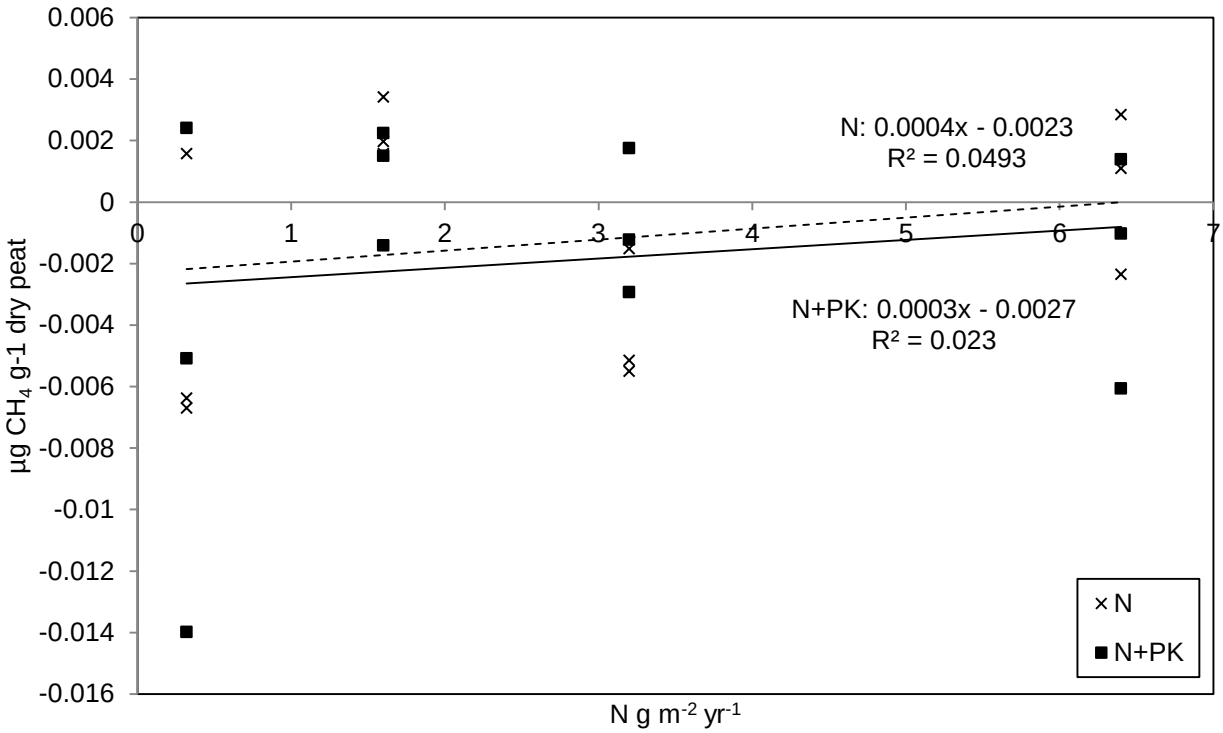

Figure 4-1 - Rates of CH4 oxidation potential across increasing N addition treatments in peat from the 30-40 cm depth horizon. Dotted line: No addition of PK; solid line: With addition of PK

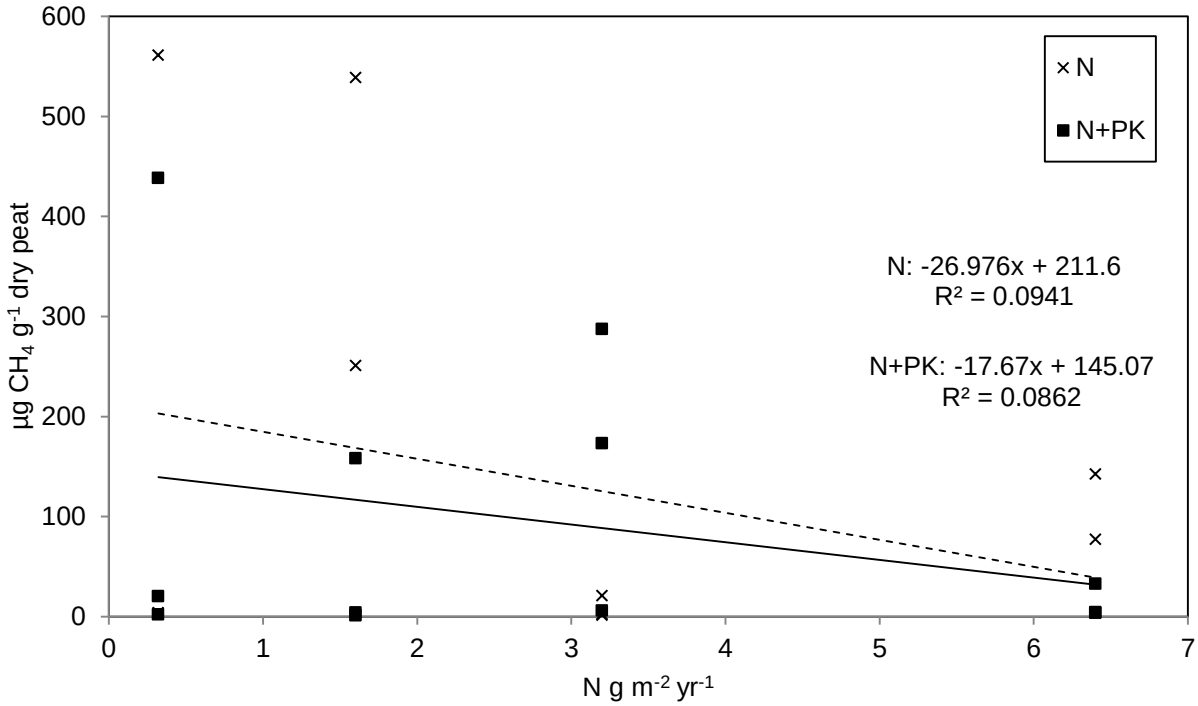

Figure 4-2 - Rates of CH4 production potential across increasing N addition treatments in peat from the 50-60 cm depth horizon. Dotted line: No addition of PK; solid line: With addition of PK

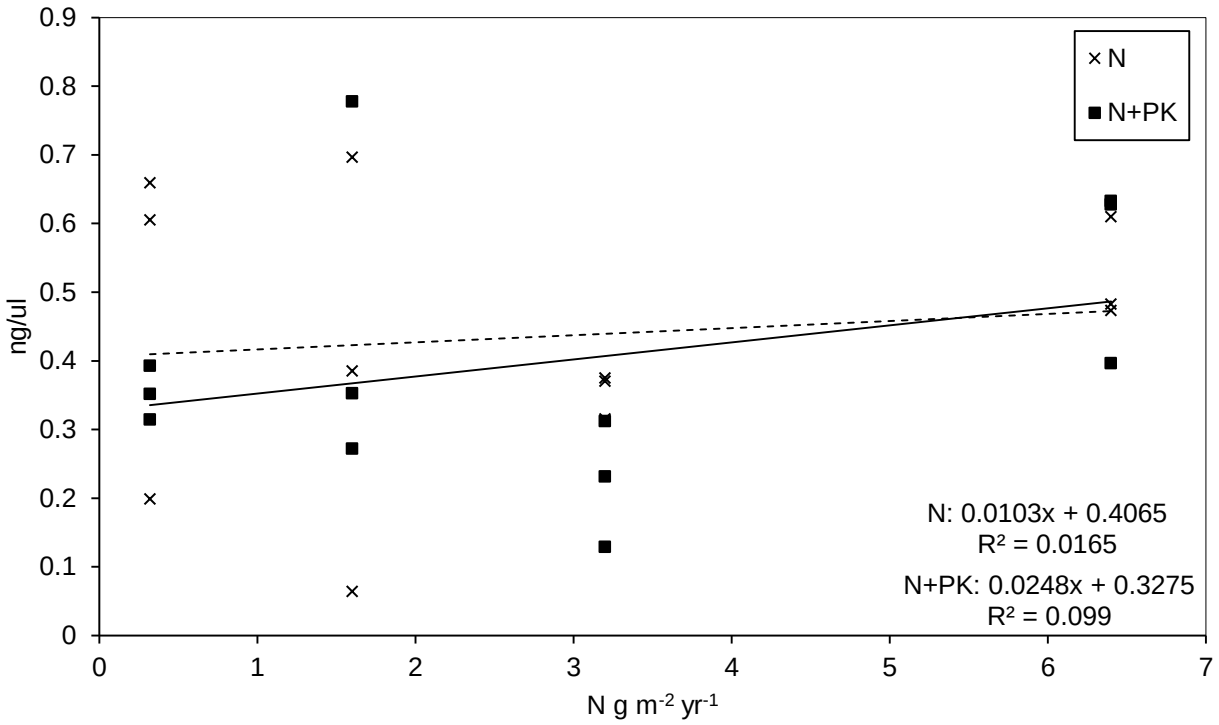

Figure 4-3 - Regression plot of N added vs *mmoX* copy numbers with and without PK. (Regression line: N only = dashed; N+PK = solid).

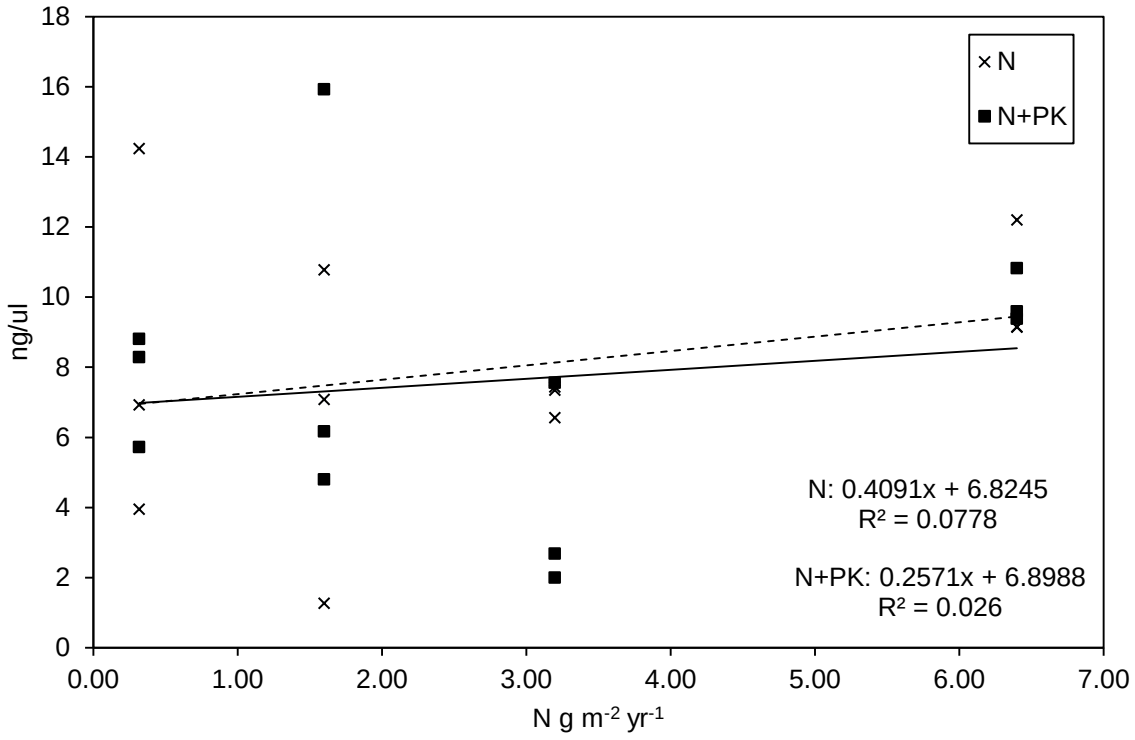

Figure 4-4 - Regression plot of N added vs *pmoA* copy number with and without PK. (Regression line: N only = dashed; N+PK = solid)

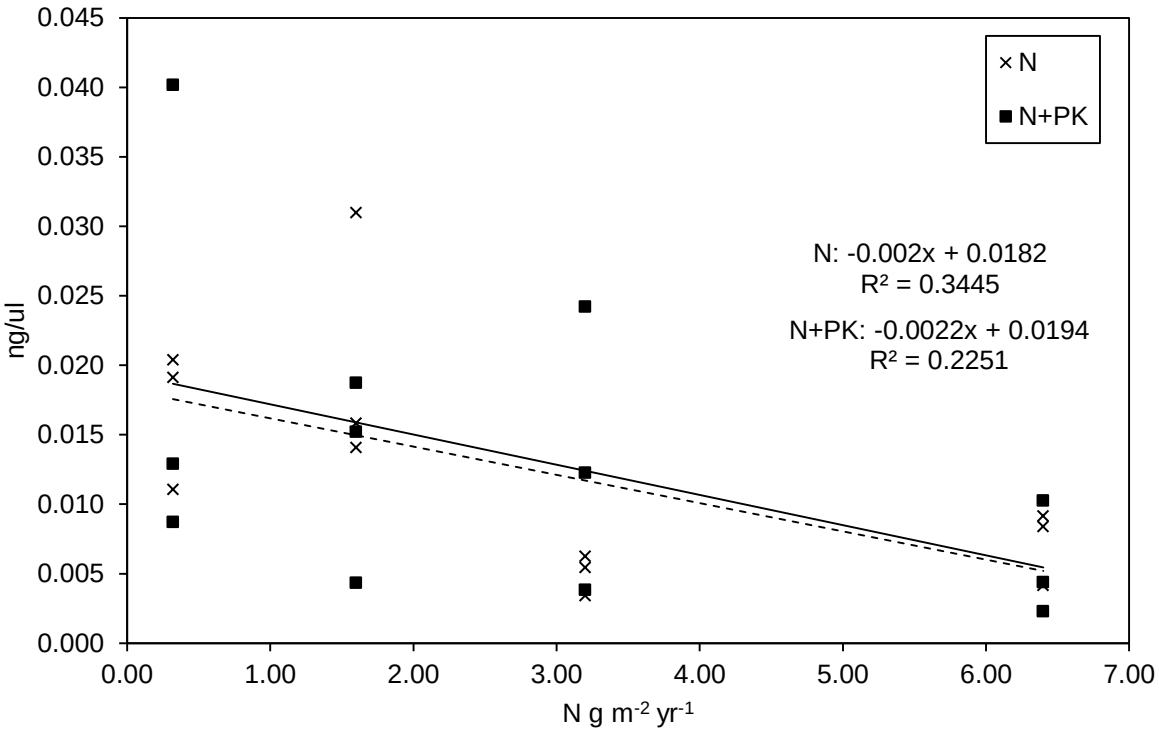

Figure 4-5 - Regression plot of N added vs *mcrA* copy number with and without PK. (Regression line: N only = dashed; N+PK = solid)

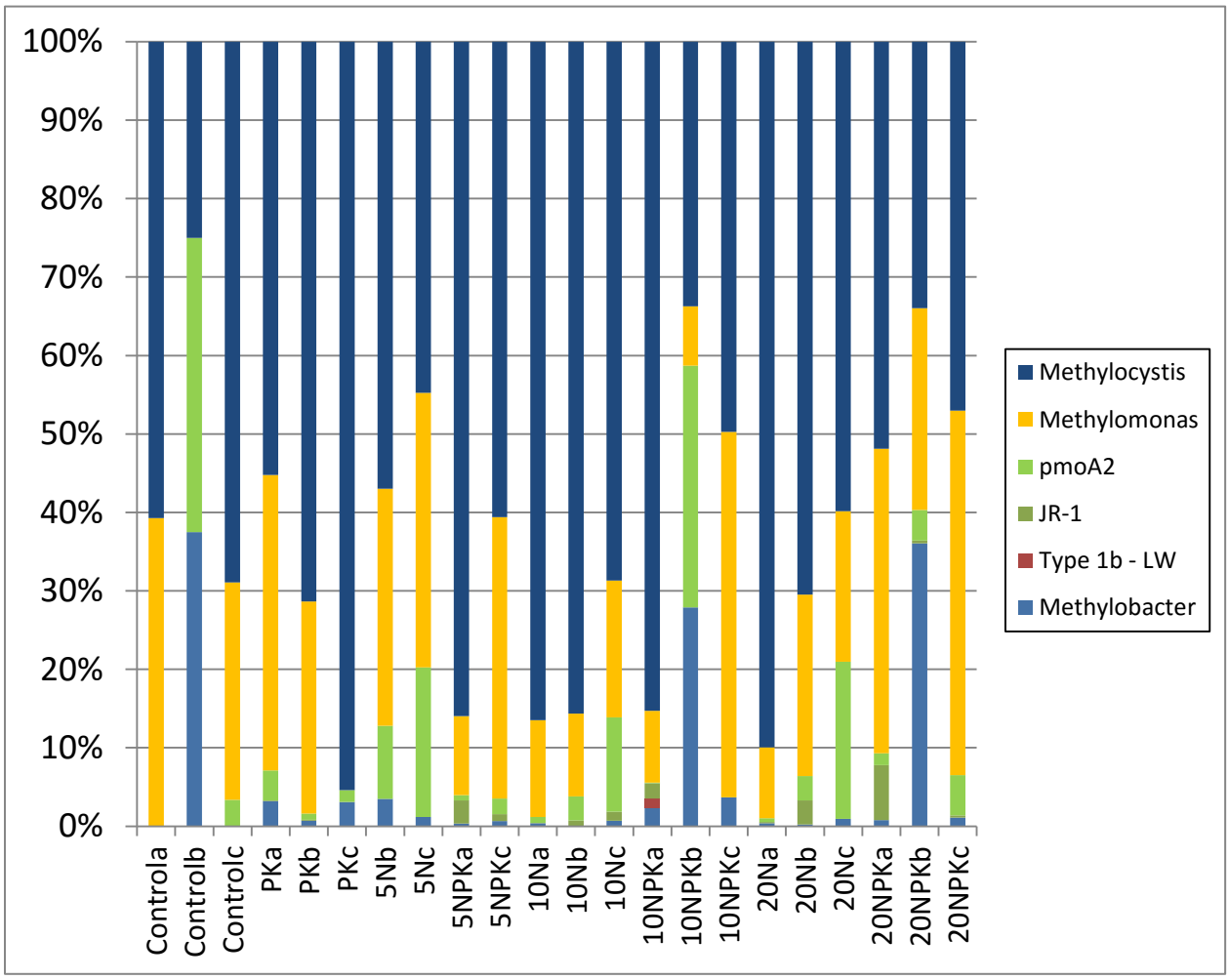

Figure 4-6 - Stacked area chart of *pmoA*-based phylogenetic groups detected in each treatment.

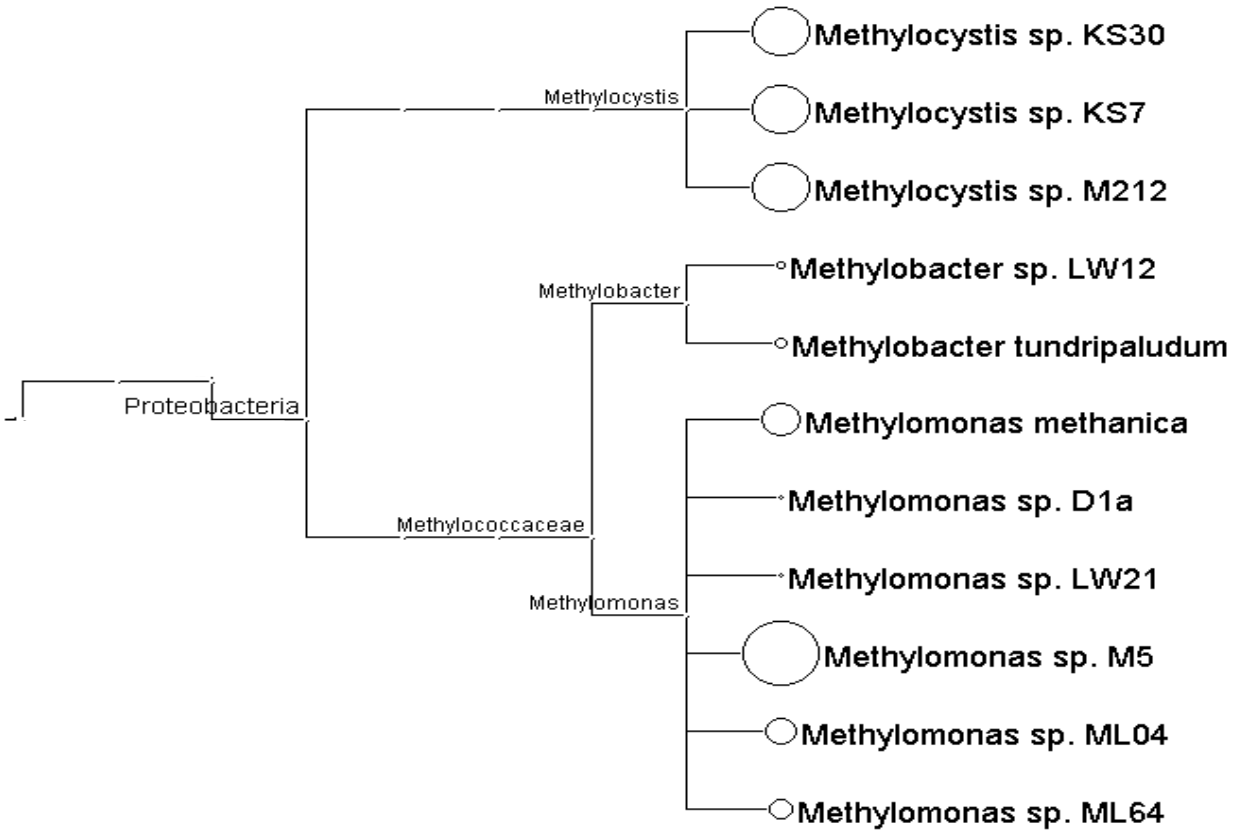

Figure 4-7 - Neighbour-joining phylogenetic tree using Bray-Curtis distance based pmoA sequences (across all treatments) obtained in this study. Circle sizes are proportional to number of OTUs

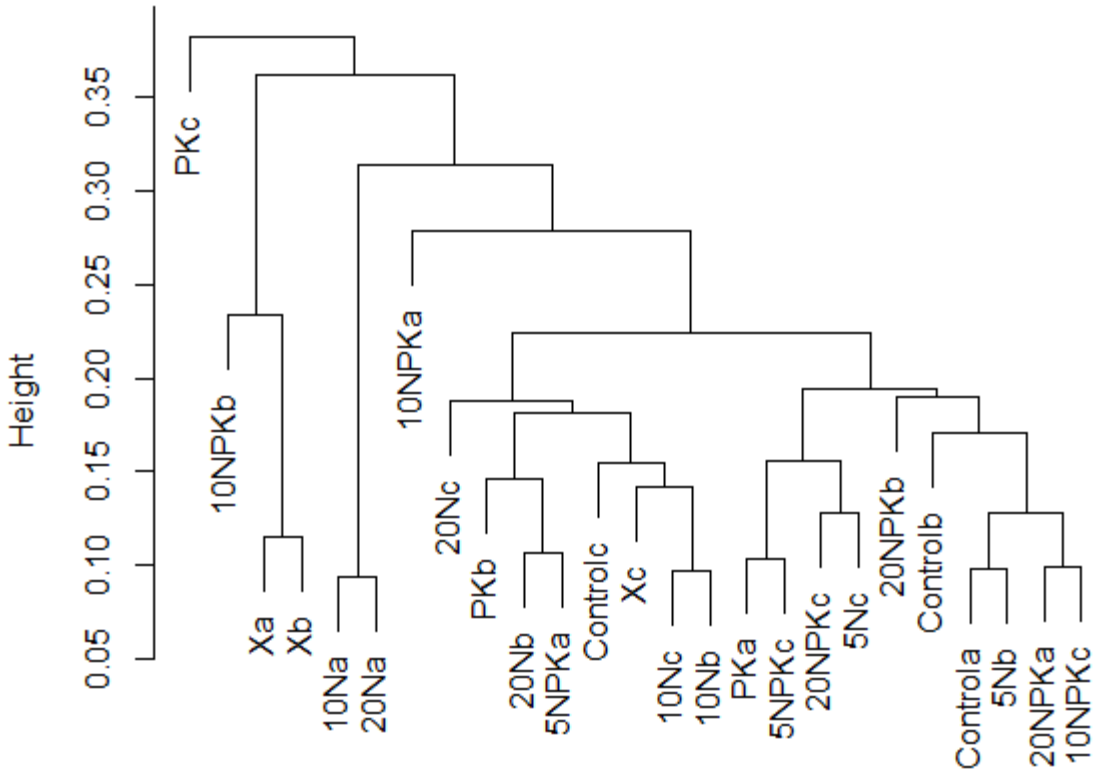

Figure 4-8 - Hierarchal clustering using Ward's methods of pmoA based methanotroph communities across all plots. No significant clustering was observed by treatment

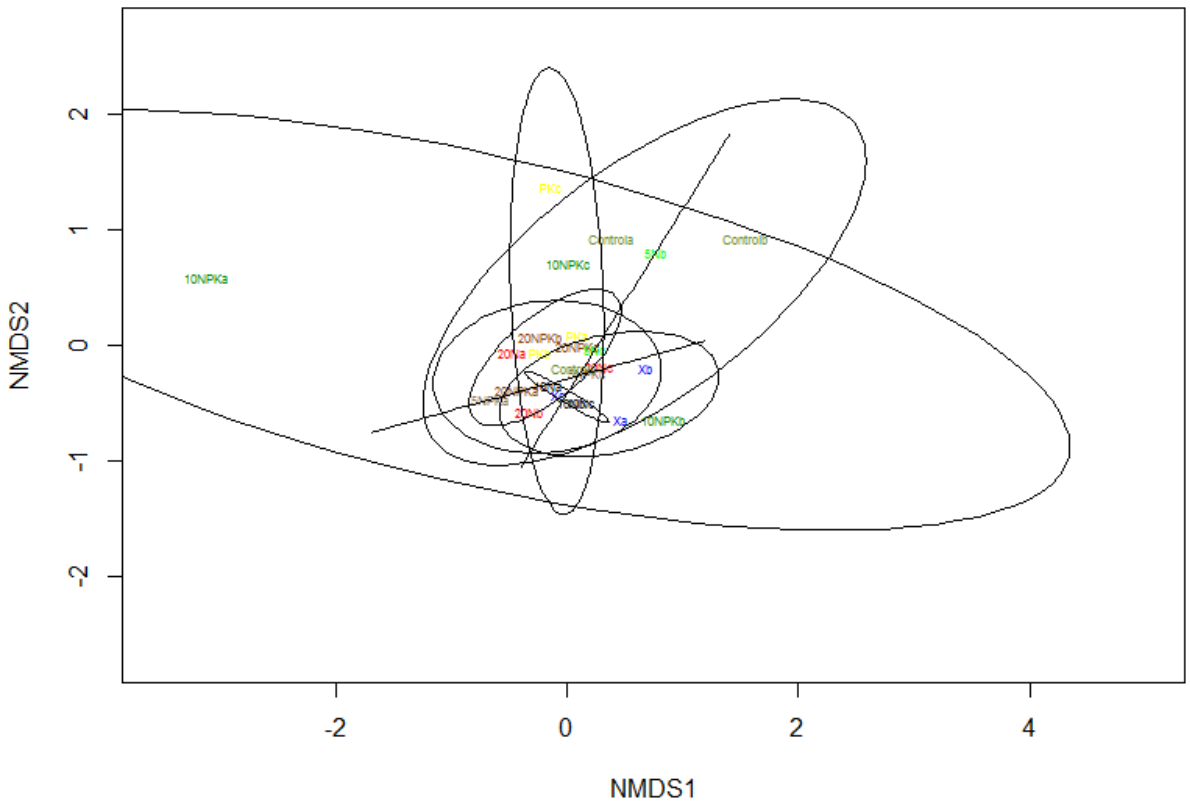

Figure 4-9 - NMDS analysis of *pmoA* based methanotroph communities. Ellipses of each treatment show 95% confidence intervals.

4.7 Tables

Table 4-1 - Primers for CH_4 dynamics functional genes

Target gene	Primer name	Sequence	Annealing (°C)	Citation
pmoA	A189F	GGN GAC TGG GAC TTC TGG	65.5	(Costello and Lidstrom, 1999)
	mb661R	CCG GMG CAA CGT CYT TAC C		
mmoX	mmoXLF	GAA GAT TGG GGC GGC ATC TG	68.0	(Rahman et al., 2011)
	mmoXLR	CCC AAT CAT CGC TGA AGG AGT		
mcrA	mlasF	GGT GGT GTM GGD TTC ACM CAR TA	48.0	(Steinberg and Regan, 2008)
	mcrA-revR	CGT TCA TBG CGT AGT TVG GRT AGT		

5. Thesis conclusions and future directions

The original questions of my thesis centred on whether and how increased N and nutrient additions in an ombrotrophic bog would impact microbial community structure, and in turn how any changes would link to microbial greenhouse gas cycling. The ultimate goal was to help understand the role microbes play in the climate change feedbacks in northern peatlands. With the continued increase of global anthropogenic inorganic N and potential negative impacts on peatland C storage, these are valid question to consider.

To answer these questions, I began with broad fingerprinting approaches to characterize microbial community structure (i.e. using T-RFLP and qPCR for fungi–bacteria ratios). My data have indicated changes in microbial communities following N and nutrient addition; and both bacterial and fungal communities have changed. These patterns were perhaps not surprising considering the impacts additions have had on the vegetation community and even on broad physical characteristics such as peat depth and relative water table position. In control plots, *Sphagnum* mosses were able to suppress microbial activities via the production of anti-microbial chemicals and by maintaining the strongly acidic conditions in surface peat. *Sphagnum* mosses perhaps were also successful at keeping numbers and biomass of higher trophic plants lower; including less rich and labile organic substrates and nutrients to the soil through leaf litter and root exudates. As microbial communities change, microbial decomposition increases simultaneously, thus increasing C export. This pattern has been observed in previous studies, including a weakened ability to store C at the ecosystem level (Basiliko et al., 2006; Larmola et al., 2013).

My data presented in this thesis do not contain detailed taxonomic information on the type of microbes at hand. Obtaining microbial "species-level" data could help provide an idea of what functional group have appeared as a result of N deposition. With current technology and the booming –omics research approaches, next-generation high throughput sequencing might be a logical next step. Alternatively, metagenomics approaches might allow a functional profile of each treatment and answer many other questions concerning fluxes of greenhouse gases, C, and nutrient cycling.

The second chapter of my thesis looked at N and nutrient addition effects on microbial CH_4 dynamics, including methanotrophs and methanogens. Peatlands are typically net C sinks but also generate more than 10% of atmospheric CH_4, which is 25 times more potent as a greenhouse gas compared to CO_2. It was initially expected that understanding how communities of the key microbial players affect CH_4 dynamics was essential in answering one of my main thesis question: what is the impact of N and nutrient addition on CH_4 cycling, methanotroph community and methanogens in an ombrotrophic bog? However my data indicated N and nutrient addition had no significant impact on methanotroph communities in contrast to prior expectations. NMDS with pyrotag sequence data and *mmoX* and *pmoA* qPCR data have shown that were no substantial community structure or methanotroph abundance differences. I was expecting a shift in methanotroph community structure considering the major shift in vegetation in the high N + PK plots in addition to the supporting data on how NH_4^+ acts as a competitive inhibitor. It would be important to obtain *in situ* field flux data to further understand the impact of N deposition on methanotrophs.

The other key microbial players in CH_4 cycling are the methanogens. In past studies, N deposition has been associated with increases atmospheric CH_4 release at Mer Bleue. However, I

have observed a significant decrease in *mcrA* gene abundance with fertilization. Additionally, data indicated no significant effect on CH_4 production potential. One explanation might have been that the vascular plants increasing in abundance provided more oxygen to the rhizosphere via aerenchyma. In doing so, the strictly anaerobic methanogens, were inhibited, consistent with the reducing *mcrA* copy numbers. As N added did not reach the depth where methanogens were studied, it is impossible to link any interference from denitrifiers to methanogens. More data on soils chemistry and physical properties could help understand the impact of N added on methanogens.

As mentioned in Roulet et al (2012), Mer Bleue bog has shown inter-annual variability of C exchange due to changes in water table levels. Both sampling years in my thesis had significantly different water tables and my data in the first research chapter indicated significant changes in broad-spectrum microbial communities between years; this important factor of inter-annual variability could also have affected CH_4 cycling communities (i.e. there might have been effects in the drier year of 2013 when methanotrophs and methanogens were not characterized).

Microbial communities are very dynamic and can adapt to environmental changes quickly. My data showed a number reasons to believe that anthropogenic N and nutrient deposition impact peatland microbial biogeochemistry and might alter the natural processes of C and greenhouse gas cycling in particular. Although they constitute only a small proportion of the Earth's land, peatlands are important C reservoirs and sources of CH_4. With the data I have presented in this thesis, I have shown that N and nutrient addition is changing peatland microbial communities potentially related to broad scale mineralization and C release that is consisted with prior *in situ* studies of C exchange Those changes are likely linked to a positive feedback loop whereby increased N associated with fossil fuel burning will lead to enhanced C loss from

peatlands, warmer climate, enhanced N mineralization in peatlands, etc... However expected changes in CH_4 cycling microbial communities were not observed in this study. Although this is tentatively good news, further work across a broader range of weather conditions and water table positions is warranted, as past and ongoing measurements of *in situ* CH_4 fluxes indicated that nutrient additions may be impacting net CH_4 exchange.

6. Reference cited

Ågren, G., Bosatta, E., and Magill, A. (2001) Combining theory and experiment to understand effects of inorganic nitrogen on litter decomposition. *Oecologia* **128**: 94–98.

Armés, C.J. (2009) Methane production, oxidation, and emissions unde simulated enhanced nutrient deposition in a northern peat bog.

Basiliko, N., Jeannotte, R., Bubier, J., and Moore, T. (2006) Nutrient Input and Carbon and Microbial Dynamics in an Ombrotrophic Bog. *Geomicrobiol. J.* **23**: 531–543.

Belova, S.E., Kulichevskaya, I.S., Bodelier, P.L.E., and Dedysh, S.N. (2013) Methylocystis bryophila sp. nov., a facultatively methanotrophic bacterium from acidic Sphagnum peat, and emended description of the genus Methylocystis (ex Whittenbury et al. 1970) Bowman et al. 1993. *Int. J. Syst. Evol. Microbiol.* **63**: 1096–104.

Berendse, F., Van Breemen, N., Rydin, Ha., Buttler, A., Heijmans, M., Hoosbeek, M.R., et al. (2001) Raised atmospheric CO2 levels and increased N deposition cause shifts in plant species composition and production in Sphagnum bogs. *Glob. Chang. Biol.* **7**: 591–598.

Bodelier, P. LE (2011) Interactions between nitrogenous fertilizers and methane cycling in wetland and upland soils. *Curr. Opin. Environ. Sustain.* **3**: 379–388.

Bodelier, P.L., Roslev, P., Henckel, T., and Frenzel, P. (2000) Stimulation by ammonium-based fertilizers of methane oxidation in soil around rice roots. *Nature* **403**: 421–4.

Bodelier, P.L.E. and Laanbroek, H.J. (2004) Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *FEMS Microbiol. Ecol.* **47**: 265–77.

Bragazza, L., Buttler, A., Habermacher, J., Brancaleoni, L., Gerdol, R., Fritze, H., et al. (2012) High nitrogen deposition alters the decomposition of bog plant litter and reduces carbon accumulation. *Glob. Chang. Biol.* **18**: 1163–1172.

Bragazza, L., Buttler, A., Siegenthaler, A., and Mitchell, E.A.D. (2009) Carbon Cycling in Northern Peatlands Baird,A.J., Belyea,L.R., Comas,X., Reeve,A.S., and Slater,L.D. (eds) American Geophysical Union, Washington, D. C.

Bragazza, L., Freeman, C., Jones, T., Rydin, H., Limpens, J., Fenner, N., et al. (2006) Atmospheric nitrogen deposition promotes carbon loss from peat bogs. *Proc. Natl. Acad. Sci. U. S. A.* **103**: 19386–19389.

Bragazza, L., Siffi, C., Iacumin, P., and Gerdol, R. (2007) Mass loss and nutrient release during litter decay in peatland: The role of microbial adaptability to litter chemistry. *Soil Biol. Biochem.* **39**: 257–267.

Brauer, S.L., Cadillo-Quiroz, H., Ward, R.J., Yavitt, J.B., and Zinder, S.H. (2010) Methanoregula boonei gen. nov., sp. nov., an acidiphilic methanogen isolated from an acidic peat bog. *Int. J. Syst. Evol. Microbiol.* **61**: 45–52.

Bubier, J., Crill, P., Mosedale, A., Frolking, S., and Linder, E. (2003) Peatland responses to varying interannual moisture conditions as measured by automatic CO 2 chambers. *Global Biogeochem. Cycles* **17**: n/a–n/a.

Bubier, J.L., Moore, T.R., and Bledzki, L. a. (2007) Effects of nutrient addition on vegetation and carbon cycling in an ombrotrophic bog. *Glob. Chang. Biol.* **13**: 1168–1186.

Caldwell, B.A., Jumpponen, A., and Trappe, J.M. (2000) Utilization of Major Detrital Substrates by Dark-Septate, Root Endophytes. *Mycologia* **92**: 230–232.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et al. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **7**: 335–6.

Cardy, D.L., Laidler, V., Salmond, G.P., and Murrell, J.C. (1991) Molecular analysis of the methane monooxygenase (MMO) gene cluster of Methylosinus trichosporium OB3b. *Mol. Microbiol.* **5**: 335–42.

Carroll, P. and Crill, P. (1997) Carbon balance of a temperate poor fen. *Global Biogeochem. Cycles* **11**: 349–356.

Chen, Y., Dumont, M.G., McNamara, N.P., Chamberlain, P.M., Bodrossy, L., Stralis-Pavese, N., and Murrell, J.C. (2008) Diversity of the active methanotrophic community in acidic peatlands as assessed by mRNA and SIP-PLFA analyses. *Environ. Microbiol.* **10**: 446–59.

Colby, J., Stirling, D.I., and Dalton, H. (1977) The soluble methane mono-oxygenase of Methylococcus capsulatus (Bath). Its ability to oxygenate n-alkanes, n-alkenes, ethers, and alicyclic, aromatic and heterocyclic compounds. *Biochem. J.* **165**: 395–402.

Costello, A.M. and Lidstrom, M.E. (1999) Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. *Appl. Environ. Microbiol.* **65**: 5066–74.

Cox, P.M., Betts, R.A., Jones, C.D., Spall, S.A., and Totterdell, I.J. (2000) Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. *Nature* **408**: 184–7.

Dalton, H. (1977) Ammonia oxidation by the methane oxidising bacterium Methylococcus capsulatus strain bath. *Arch. Microbiol.* **114**: 273–279.

Dedysh, S.N., Belova, S.E., Bodelier, P.L.E., Smirnova, K. V, Khmelenina, V.N., Chidthaisong, A., et al. (2007) Methylocystis heyeri sp. nov., a novel type II methanotrophic bacterium

possessing “signature” fatty acids of type I methanotrophs. *Int. J. Syst. Evol. Microbiol.* **57**: 472–9.

Dedysh, S.N., Derakshani, M., and Liesack, W. (2001) Detection and Enumeration of Methanotrophs in Acidic Sphagnum Peat by 16S rRNA Fluorescence In Situ Hybridization, Including the Use of Newly Developed Oligonucleotide Probes for Methylocella palustris. *Appl. Environ. Microbiol.* **67**: 4850–4857.

Dedysh, S.N., Knief, C., and Dunfield, P.F. (2005) Methylocella species are facultatively methanotrophic. *J. Bacteriol.* **187**: 4665–70.

Degelmann, D.M., Borken, W., Drake, H.L., and Kolb, S. (2010) Different atmospheric methane-oxidizing communities in European beech and Norway spruce soils. *Appl. Environ. Microbiol.* **76**: 3228–35.

Deng, Y., Cui, X., Lüke, C., and Dumont, M.G. (2013) Aerobic methanotroph diversity in Riganqiao peatlands on the Qinghai-Tibetan Plateau. *Environ. Microbiol. Rep.* **5**: 566–74.

Dumont, M.G., Lüke, C., Deng, Y., and Frenzel, P. (2014) Classification of pmoA amplicon pyrosequences using BLAST and the lowest common ancestor method in MEGAN. *Front. Microbiol.* **5.**:

Dunfield, P. and Knowles, R. (1995) Kinetics of Inhibition of Methane Oxidation by Nitrate, Nitrite, and Ammonium in a Humisol. *Appl. Envir. Microbiol.* **61**: 3129–3135.

Edgar, R.C. (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**: 2460–1.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., and Knight, R. (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**: 2194–200.

Fierer, N., Jackson, J.A., Vilgalys, R., and Jackson, R.B. (2005) Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Appl. Environ. Microbiol.* **71**: 4117–20.

Fog, K. (1988) The effect of added nitrogen on the rate of decomposition of organic matter. *Biol. Rev.* **63**: 433–462.

Freitag, T.E. and Prosser, J.I. (2009) Correlation of methane production and functional gene transcriptional activity in a peat soil. *Appl. Environ. Microbiol.* **75**: 6679–87.

Fumoto, T., Kobayashi, K., Li, C., Yagi, K., and Hasegawa, T. (2007) Revising a process-based biogeochemistry model (DNDC) to simulate methane emission from rice paddy fields under various residue management and fertilizer regimes. *Glob. Chang. Biol.* **14**: 382–402.

Galloway, J.N. and Cowling, E.B. (2009) Reactive Nitrogen and The World: 200 Years of Change.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., et al. (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* **320**: 889–92.

Gilbert, B., McDonald, I.R., Finch, R., Stafford, G.P., Nielsen, A.K., and Murrell, J.C. (2000) Molecular Analysis of the pmo (Particulate Methane Monooxygenase) Operons from Two Type II Methanotrophs. *Appl. Environ. Microbiol.* **66**: 966–975.

Gorham, E. (1991) Northern Peatlands: Role in the Carbon Cycle and Probable Responses to Climatic Warming. *Ecol. Appl.* **1**: 182–195.

Gunnarsson, U., Boresejö Bronge, L., Rydin, H., and Ohlson, M. (2008) Near-zero recent carbon accumulation in a bog with high nitrogen deposition in SW Sweden. *Glob. Chang. Biol.* **14**: 2152–2165.

Hakemian, A.S. and Rosenzweig, A.C. (2007) The biochemistry of methane oxidation. *Annu. Rev. Biochem.* **76**: 223–41.

Hanson, R.S. and Hanson, T.E. (1996) Methanotrophic bacteria. *Microbiol. Rev.* **60**: 439–71.

Hasselquist, N.J., Metcalfe, D.B., and Högberg, P. (2012) Contrasting effects of low and high nitrogen additions on soil CO 2 flux components and ectomycorrhizal fungal sporocarp production in a boreal forest. *Glob. Chang. Biol.* **18**: 3596–3605.

Henckel, T., Roslev, P., and Conrad, R. (2000) Effects of O2 and CH4 on presence and activity of the indigenous methanotrophic community in rice field soil. *Environ. Microbiol.* **2**: 666–679.

Holmes, A.J., Roslev, P., McDonald, I.R., Iversen, N., Henriksen, K., and Murrell, J.C. (1999) Characterization of Methanotrophic Bacterial Populations in Soils Showing Atmospheric Methane Uptake. *Appl. Envir. Microbiol.* **65**: 3312–3318.

Imlay, J.A. (2002) How oxygen damages microbes: oxygen tolerance and obligate anaerobiosis. *Adv. Microb. Physiol.* **46**: 111–53.

IPCC (2013) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change Stocker,T.F., Qin,D., Plattner,G.-K., Tignor,M., Allen,S.K., Boschung,J., Nauels,A., Xia,Y., Bex,V., and Midgley,P.M. (eds) Cambridge University Press, United Kingdom and New York, NY, USA.

Ishii, S., Kadota, K., and Senoo, K. (2009) Application of a clustering-based peak alignment algorithm to analyze various DNA fingerprinting data. *J. Microbiol. Methods* **78**: 344–50.

Jassey, V.E.J., Gilbert, D., Binet, P., Toussaint, M.-L., and Chiapusio, G. (2011) Effect of a temperature gradient on Sphagnum fallax and its associated living microbial communities: a study under controlled conditions. *Can. J. Microbiol.* **57**: 226–35.

Juutinen, S., Bubier, J.L., and Moore, T.R. (2010) Responses of Vegetation and Ecosystem CO2 Exchange to 9 Years of Nutrient Addition at Mer Bleue Bog. *Ecosystems* **13**: 874–887.

Kaplan, C.W. and Kitts, C.L. (2003) Variation between observed and true Terminal Restriction Fragment length is dependent on true TRF length and purine content. *J. Microbiol. Methods* **54**: 121–5.

Keith, J. and Dillon, P. (1989) Acidic precipitation research in Canada. In, Bresser,A. and Salomons,W. (eds), *Acidic Precipitation: International Overview and Assessment.* Springer-Verlag, New York, pp. 1–40.

Kip, N., Dutilh, B.E., Pan, Y., Bodrossy, L., Neveling, K., Kwint, M.P., et al. (2011) Ultra-deep pyrosequencing of pmoA amplicons confirms the prevalence of Methylomonas and Methylocystis in Sphagnum mosses from a Dutch peat bog. *Environ. Microbiol. Rep.* **3**: 667–73.

Klüber, H.D. and Conrad, R. (1998) Effects of nitrate, nitrite, NO and N2O on methanogenesis and other redox processes in anoxic rice field soil. *FEMS Microbiol. Ecol.* **25**: 301–318.

Kolb, S., Knief, C., Stubner, S., and Conrad, R. (2003) Quantitative Detection of Methanotrophs in Soil by Novel pmoA-Targeted Real-Time PCR Assays. *Appl. Environ. Microbiol.* **69**: 2423–2429.

Lamers, L.P.M., Bobbink, R., and Roelofs, J.G.M. (2000) Natural nitrogen filter fails in polluted raised bogs. *Glob. Chang. Biol.* **6**: 583–586.

Larmola, T., Bubier, J.L., Kobyljanec, C., Basiliko, N., Juutinen, S., Humphreys, E., et al. (2013) Vegetation feedbacks of nutrient addition lead to a weaker carbon sink in an ombrotrophic bog. *Glob. Chang. Biol.* **19**: 3729–3739.

Larmola, T., Tuittila, E.-S., Tiirola, M., Nykänen, H., Martikainen, P.J., Yrjälä, K., et al. (2010) The role of Sphagnum mosses in the methane cycling of a boreal mire. *Ecology* **91**: 2356–2365.

LeBauer, D.S. and Treseder, K.K. (2008) Nitrogen Limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* **89**: 371–379.

Lilleskov, E.A., Fahey, T.J., Horton, T.R., and Lovett, G.M. (2002) Belowground Ectomycorrhizal Fungal Community Change Over a Nitrogen Deposition Gradient in Alaska. *Ecology* **83**: 104–115.

Lilleskov, E.A., Hobbie, E.A., and Horton, T.R. (2011) Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. *Fungal Ecol.* **4**: 174–183.

Limpens, J. and Berendse, F. (2003) How litter quality affects mass loss and N loss from decomposing Sphagnum. *Oikos* **103**: 537–547.

Limpens, J., Granath, G., Gunnarsson, U., Aerts, R., Bayley, S., Bragazza, L., et al. (2011) Climatic modifiers of the response to nitrogen deposition in peat-forming Sphagnum mosses: a meta-analysis. *New Phytol.* **191**: 496–507.

Lukow, T., Dunfield, P., and Liesack, W. (2000) Use of the T-RFLP technique to assess spatial and temporal changes in the bacterial community structure within an agricultural soil planted with transgenic and non-transgenic potato plants. *FEMS Microbiol. Ecol.* **32**: 241–247.

Luthon, P.E., Wayne, J.M., Sharp, R.J., and Riley, P.W. (2002) The mcrA gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfillb. *Microbiology* **148.**:

Malmer, N., Albinsson, C., Svensson, B.M., and Wallen, B. (2003) Interferences between Sphagnum and vascular plants: effects on plant community structure and peat formation. *Oikos* **100**: 469–482.

Manzoni, S., Jackson, R.B., Trofymow, J.A., and Porporato, A. (2008) The global stoichiometry of litter nitrogen mineralization. *Science* **321**: 684–6.

McDonald, I.R., Bodrossy, L., Chen, Y., and Murrell, J.C. (2008) Molecular ecology techniques for the study of aerobic methanotrophs. *Appl. Environ. Microbiol.* **74**: 1305–15.

McMurdie, P.J. and Holmes, S. (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* **8**: e61217.

Moore, T.R. and Basiliko, N. (2006) Decomposition in boreal peatlands. In, Wieder,R.K. and Vitt,D.H. (eds), *Boreal Peatland Ecosystems*, Ecological Studies. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 125–143.

Moore, T.R. and Knowles, R. (1989) The Influence of Water Table Levels on Methane and Carbon Dioxide Emissions From Peatland Soils. *Can. J. Soil Sci.* **69**: 33–38.

Moore, T.R., Young, A., Bubier, J.L., Humphreys, E.R., Lafleur, P.M., and Roulet, N.T. (2011) A Multi-Year Record of Methane Flux at the Mer Bleue Bog, Southern Canada. *Ecosystems* **14**: 646–657.

Murphy, M.T. and Moore, T.R. (2010) Linking root production to aboveground plant characteristics and water table in a temperate bog. *Plant Soil* **336**: 219–231.

Murrell, J.C., Gilbert, B., and McDonald, I.R. (2000) Molecular biology and regulation of methane monooxygenase. *Arch. Microbiol.* **173**: 325–332.

Murrell, J.C., McDonald, I.R., and Bourne, D.G. (1998) Molecular methods for the study of methanotroph ecology. *FEMS Microbiol. Ecol.* **27**: 103–114.

Myers, B., Webster, K.L., Mclaughlin, J.W., and Basiliko, N. (2012) Microbial activity across a boreal peatland nutrient gradient: the role of fungi and bacteria. *Wetl. Ecol. Manag.* **20**: 77–88.

Nyerges, G., Han, S.-K., and Stein, L.Y. (2010) Effects of ammonium and nitrite on growth and competitive fitness of cultivated methanotrophic bacteria. *Appl. Environ. Microbiol.* **76**: 5648–51.

Nyerges, G. and Stein, L.Y. (2009) Ammonia cometabolism and product inhibition vary considerably among species of methanotrophic bacteria. *FEMS Microbiol. Lett.* **297**: 131–6.

Oksanen, J., Blanchet, F.G., Kindt, Roeland Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., et al. (2014) vegan: Community Ecology Package.

Pfaffl, M.W. (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* **29**: 45e–45.

Preston, M.D., Smemo, K.A., McLaughlin, J.W., and Basiliko, N. (2012) Peatland microbial communities and decomposition processes in the james bay lowlands, Canada. *Front. Microbiol.* **3**: 70.

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rahman, M.T., Crombie, A., Chen, Y., Stralis-Pavese, N., Bodrossy, L., Meir, P., et al. (2011) Environmental distribution and abundance of the facultative methanotroph Methylocella. *ISME J.* **5**: 1061–6.

Roulet, N.T., Nilsson, M.B., and Humphreys, E.R. (2012) Peatland wetness and the inter-annual variability of net ecosystem exchange: comparison of multi-year records from two different types of northern peatlands. *EGU Gen. Assem. 2012*.

Roulet, N., Lafleur, P.M., Richard, P.J.H., Moore, T.R., Humphreys, E.R., and Bubier, J.L. (2007) Contemporary carbon balance and late Holocene carbon accumulation in a northern peatland. *Glob. Chang. Biol.* **13**: 397–411.

Rydin, H. and Clymo, R.S. (1989) Transport of Carbon and Phosphorus Compounds about Sphagnum. *Proc. R. Soc. B Biol. Sci.* **237**: 63–84.

Rydin, H. and Jeglum, J.K. (2013) The Biology of Peatlands 2e ed. OUP Oxford.

Schnell, S. and King, G.M. (1996) Responses of methanotrophic activity in soils and cultures to water stress. *Appl. Environ. Microbiol.* **62**: 3203–9.

Selosse, M.-A., Setaro, S., Glatard, F., Richard, F., Urcelay, C., and Weiss, M. (2007) Sebacinales are common mycorrhizal associates of Ericaceae. *New Phytol.* **174**: 864–78.

Semrau, J.D., DiSpirito, A.A., and Yoon, S. (2010) Methanotrophs and copper. *FEMS Microbiol. Rev.* **34**: 496–531.

Semrau, J.D., Jagadevan, S., Dispirito, A.A., Khalifa, A., Scanlan, J., Bergman, B.H., et al. (2013) Methanobactin and MmoD work in concert to act as the "copper-switch" in methanotrophs. *Environ. Microbiol.*

Semrau, J.D., Zolandz, D., Lidstrom, M.E., and Chan, S.I. (1995) The role of copper in the pMMO of Methylococcus capsulatus bath: a structural vs. catalytic function. *J. Inorg. Biochem.* **58**: 235–44.

Shannon, R.D. and White, J.R. (1996) Methane efflux from emergent vegetation in peatlands. *J. Ecol.* **84**: 239.

Sisterson, D., Bowersox, V., and Olsen, A. (1994) A review of spatial and temporal trends of precipitation composition for North America for the period 1979– 1987. In Rose J, editor. Acid Rain, Current Situation and Remedies In Rose J (ed) Gordon and Breach Science Publishers, Yverdon, Switzerland.

Steinberg, L.M. and Regan, J.M. (2009) mcrA-targeted real-time quantitative PCR method to examine methanogen communities. *Appl. Environ. Microbiol.* **75**: 4435–42.

Steinberg, L.M. and Regan, J.M. (2008) Phylogenetic comparison of the methanogenic communities from an acidic, oligotrophic fen and an anaerobic digester treating municipal wastewater sludge. *Appl. Environ. Microbiol.* **74**: 6663–71.

Steudler, P.A., Bowden, R.D., Melillo, J.M., and Aber, J.D. (1989) Influence of nitrogen fertilization on methane uptake in temperate forest soils. *Nature* **341**: 314–316.

Stolyar, S., Costello, A.M., Peeples, T.L., and Lidstrom, M.E. (1999) Role of multiple gene copies in particulate methane monooxygenase activity in the methane-oxidizing bacterium Methylococcus capsulatus Bath. *Microbiology* **145**: 1235–1244.

Strickland, M.S. and Rousk, J. (2010) Considering fungal:bacterial dominance in soils – Methods, controls, and ecosystem implications. *Soil Biol. Biochem.* **42**: 1385–1395.

Thauer, R.K. (1998a) Biochemistry of methanogenesis: a tribute to Marjory Stephenson:1998 Marjory Stephenson Prize Lecture. *Microbiology* **144**: 2377–2406.

Thauer, R.K. (1998b) Biochemistry of methanogenesis: a tribute to Marjory Stephenson:1998 Marjory Stephenson Prize Lecture. *Microbiology* **144**: 2377–2406.

Tiedje, J.M., Asuming-Brempong, S., Nüsslein, K., Marsh, T.L., and Flynn, S.J. (1999) Opening the black box of soil microbial diversity. *Appl. Soil Ecol.* **13**: 109–122.

Treseder, K.K. (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecol. Lett.* **11**: 1111–20.

Turunen, J., Roulet, N.T., Moore, T.R., and Richard, P.J.H. (2004) Nitrogen deposition and increased carbon accumulation in ombrotrophic peatlands in eastern Canada. *Global Biogeochem. Cycles* **18.**:

Turunen, J., Tomppo, E., Tolonen, K., and Reinikainen, A. (2002) Estimating carbon accumulation rates of undrained mires in Finland – application to boreal and subarctic regions. *The Holocene* **12**: 69–80.

Vorobev, A., Jagadevan, S., Baral, B.S., Dispirito, A.A., Freemeier, B.C., Bergman, B.H., et al. (2013) Detoxification of mercury by methanobactin from Methylosinus trichosporium OB3b. *Appl. Environ. Microbiol.* **79**: 5918–26.

Waddington, J.M. and Roulet, N.T. (1996) Atmosphere-wetland carbon exchanges: Scale dependency of CO 2 and CH 4 exchange on the developmental topography of a peatland. *Global Biogeochem. Cycles* **10**: 233–245.

Wang, M., Moore, T.R., Talbot, J., and Richard, P.J.H. (2014) The cascade of C:N:P stoichiometry in an ombrotrophic peatland: from plants to peat. *Environ. Res. Lett.* **9**: 024003.

Wang, M., Murphy, M.T., and Moore, T.R. (2014) Nutrient resorption of two evergreen shrubs in response to long-term fertilization in a bog. *Oecologia* **174**: 365–77.

Williams, C.J., Shingara, E.A., and Yavitt, J.B. (2000) Phenol oxidase activity in peatlands in New York State: Response to summer drought and peat type. *Wetlands* **20**: 416–421.

Van Winden, J.F., Reichart, G.-J., McNamara, N.P., Benthien, A., and Damsté, J.S.S. (2012) Temperature-induced increase in methane release from peat bogs: a mesocosm experiment. *PLoS One* **7**: e39614.

Yavitt, J.B. (2000) Nutrient Dynamics of Soil Derived from Different Parent Material on Barro Colorado Island, Panama1. *Biotropica* **32**: 198–207.